Strategic management of chicory (*Cichorium intybus* L.) for improved milk quality and nitrogen utilization in dairy farm systems

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Mancoba Christopher Mangwe

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As consumers interest in the environment and the source and quality of their functional food increase, milk quality from pastoral grazed systems is becoming increasingly important. Additionally, strategies to diminish the loss of nitrogen (**N**) associated with the traditional perennial ryegrass/white clover [*Lolium perenne* L./*Trifolium repens* L.; (**RGWC**)] are being considered. Recent research has identified the value of forage herbs as a pathway to mitigate N losses in the environment. However, the current research has focused on the role of plantain (*Plantago lanceolata* L.) with little emphasis on the alternative herb chicory (*Cichorium intybus* L.). Chicory is known to have high palatability and healthy fatty acid (**FA**) profile, but little is known about the best management practices for chicory in an irrigated dairy environment or about the animal response in relation to milk quality and N partitioning. The main aim of the thesis was to investigate the effects chicory-based herbage has on urine N excretion, milk production and milk FA composition of dairy cows, as well as to understand the mechanisms leading to the variation in milk FA of cows grazing chicory-based herbage and those grazing RGWC-based herbage. A secondary aim was to investigate the effect of grazing management (defoliation intensity, severity and timing) before and after vernalisation on morphology, functional traits, herbage DM production and biochemical composition of chicory and the subsequent effect on milk

production and milk FA composition. Two grazing experiments and one agronomy experiment were conducted on irrigated chicory-based pastures grazed by dairy cows.

The first experiment compared milk production, milk FA, urination patterns, and N use efficiency of cows grazing chicory, plantain, or RGWC. Fifty-four Friesian x Jersey cows in late lactation were blocked into replicated groups of six cows and offered one of three pasture types. Automated urine sensors measuring urine volume and timing were affixed to approximately 10 random cows per treatment for 24 h. Milk yield was similar for all treatments, but milk solids production (protein + fat) were greater from cows grazing chicory than RGWC (1.86 vs 1.72 kg MS/cow.day, $P < 0.01$) while plantain was intermediate (1.76 kg MS/cow.day). Milk produced from cows grazing chicory or plantain contained greater proportions of omega-3 FA than that from cows on RGWC ($P < 0.01$), despite lower omega-3 FA in herbage. The traditional RGWC increased the percentage of conjugated linoleic acid (**CLA;** isomer C18:2 c9 t11) and vaccenic acids in milk compared with those of milk produced from herbs ($P < 0.01$). This reflected the greater percentage of α-linolenic acid in RGWC ($P = 0.02$) than in forage herbs. There was large variation in urine event volume (0.13–11.6 L per event), though mean urine volume per event was similar for each treatment (3.01 ± 0.3 L per event; $P = 0.24$). Water, sodium and potassium intakes from herbage was greatest for chicory compared with plantain or RGWC. Frequency of urination increased with increasing water, sodium and potassium intakes and cows grazing chicory and plantain urinated more often (28.6 and 21.2 events/cow.day, respectively) than those grazing RGWC (13.9 events/ cow.day, $P < 0.05$). Consequently, cows grazing chicory excreted 1.5 and 2.4 times more urine per day ($P < 0.01$) than cows grazing plantain and RGWC, respectively. Apparent N intake was similar between cows grazing chicory and RGWC, but greater than cows grazing plantain. However, urinary N (**UN**) concentrations from cows grazing chicory and plantain were similar ($P > 0.05$), but substantially lower than RGWC (1.3 vs 4.8 g/kg; $P < 001$). The reduction in UN in cows grazing herbs was mainly attributed to increased number of urinations, which resulted in urine dilution. The combination of similar urine volume per event in all three forages, frequent urinations and substantially lower UN concentration in cows grazing the forage herbs indicate a benefit from forage herbs to reduce N load onto pasture. Overall, the findings from this first experiment demonstrated the potential benefit of chicory to alter milk FA composition, however, the mechanisms leading to the increase in beneficial FA in milk of cows offered the chicory were not clear and needed further investigations. Additionally, while the study demonstrated that sole diets of chicory elevated urination frequency and reduced UN concentration when compared with RGWC, practically however, it is unlikely that farmers will grow large areas of their farm in chicory and therefore more strategic management of chicory to alter animal response (milk production, milk FA composition and urine N excretion patterns) warranted investigation.

The second grazing experiment investigated the effects of including chicory into the traditional feeding regime of ryegrass/white clover, and time of its allocation on milk production, rumen fermentation, and FA composition of milk and rumen digesta of dairy cows. Nine groups of four cows were allocated one of three replicated feeding regimes: (1) RGWC, (2) RGWC + morning allocation of chicory (**CHAM**), and (3) RGWC + afternoon allocation of chicory (**CHPM**). One cow per group had a rumen cannulae fitted. Treatment did not affect total grazing time or estimated dry matter intake, but cows ruminated less when fed chicory than RGWC. Allocating chicory in the afternoon elevated milk production compared with RGWC and CHAM. Similar to the first grazing experiment, milk from cows grazing chicory contained greater concentrations of polyunsaturated FA (**PUFA**) such as C18:3 c9, 12, 15 and C18:2 c9, 12 than those on RGWC. As with milk, rumen digesta concentration of PUFA increased when cows grazed on chicory rather than RGWC, which corresponded with lower concentrations of intermediate vaccenic and biohydrogenation end-product stearic acid for cows grazing on chicory. Mean ruminal pH was lower for cows offered chicory than those on RGWC, reflecting greater rumen concentrations of volatile fatty acids (**VFA**) for cows fed chicory. Apparent N intake was unaffected by treatment ($P = 0.151$). Chicory inclusion increased urination frequency by up to 69%, resulting in a 33% mean decline in UN concentration, independently of time of allocation. A decline in UN concentration was detected following greater urination activity, with cows offered CHAM having lower UN concentration than cows offered CHPM or RGWC ($P \leq 0.05$) at 2000 h. The milk FA and urine excretion results of this second grazing experiment were consistent with the previous experiment showing that strategic use of chicory can achieve desired outcomes. The basis for grazing management decisions in this second experiment was informed by detailed agronomic investigation of chicory.

Concurrent agronomy studies of chicory were conducted alongside the grazing experiments. The agronomy experiment measured functional traits, morphology, herbage production and biochemical composition of chicory under irrigated field conditions before and after vernalisation in Canterbury, New Zealand. The experimental site was laid out in a complete randomized block design with four replications where two regrowth intervals and two defoliation heights were applied. Regrowth interval had a stronger influence over functional traits and herbage production than defoliation height, with more pronounced effects after vernalisation. Plants managed under shorter regrowth intervals had narrower roots with lower concentration of sugars than plants under longer intervals, which might compromise their longevity. In addition, plants managed under shorter intervals remained mostly vegetative with heavier and longer leaves, though with reduced photosynthetic capacity than those managed under longer intervals. The thermal time to initiate stem elongation in plants managed under longer intervals was ~274 growing degree-days, with a mean stem elongation rate increasing linearly at 1.4 ± 0.08 mm/growing degree-days. In terms of nutrient and FA composition of chicory herbage, the findings showed significant interaction effects between phenology stage and regrowth interval. Before vernalisation, the FA and nutrient components exhibited little variation with regrowth interval and defoliation height irrespective of time of day. After vernalisation, concentrations of linoleic, linolenic and total FA declined, by 50, 74 and 63% respectively. Furthermore, extending the regrowth interval from 300 to 600 growing degree-days after vernalisation caused a decline of 28% of linoleic, 40% of linolenic and 33% of total FA concentrations. Generally, there were little to no diurnal changes in the main FA's with the exception of linoleic and oleic acids after vernalisation ($P < 0.01$). Vernalisation decreased CP, digestibility and ME, while fibre contents increased. Between morning and afternoon there were general declines in CP and fibre contents as well as increases in soluble sugars and non-fibre carbohydrates of the herbage regardless of vernalisation. While feed quality was generally poorer in vernalised chicory, the concentration of polyunsaturated FA and digestibility can be enhanced by shortening the regrowth interval. The increase in FA and feeding value in afternoon herbage may enhance both productivity and quality of the milk while minimizing environmental risks associated with pastoral farming.

Overall, the thesis shows, for the first time, that chicory is a high moisture forage crop that increases urination frequency and dilutes the N concentration of the urine when included into that traditional grazing regime with RGWC. This demonstrates the potential role of chicory in promoting environmentally sustainable pastoral dairying systems. Timing of chicory allocation affected the diurnal patterns of urine excretion, but not the daily urine excretion output, indicating that chicory could be allocated anytime during the day without compromising the potential environmental benefits. Including chicory into a traditional grazing regime has the potential to increase milk production of dairy cows and allocating chicory during the afternoon is a useful strategy that can translate to improved milk production. The thesis further confirmed that feeding chicory to dairy cows enhanced the concentration of functional FA in milk. The lower rumen pH, lower concentration of biohydrogenation intermediate and end product vaccenic and stearic acids, respectively, and the elevated concentration of PUFA in the rumen of cows fed chicory suggested reduced biohydrogenation and may explain the elevated concentration of PUFA in the milk of cows fed chicory compared with those fed RGWC. In terms of managing chicory pastures, the experiments conducted demonstrated the potential trade-offs between herbage production and feeding value as chicory plants exposed to longer regrowth intervals accumulated larger amounts of aerial mass and reduced the highly nutritious leaf proportion of the herbage. Alternating frequent and infrequent defoliation regimes might be used to optimise vegetative growth, root reserves, and pasture persistence. A key finding from the thesis quantified the growing degree-days to initiate stem elongation post vernalisation, which provides management directive for timing of defoliation of chicory in order to maintain feed quality for grazing livestock.

List of publications by Candidate

Published journal articles

Mangwe, M.C., Bryant, H., Beck, M.R., Beale, N., Bunt, C., Gregorini, P. (2019). Forage herbs as an alternative to ryegrass-white clover to alter urination patterns in grazing dairy systems. *Animal Feed Science and Technology* 252; 11–22.

Mangwe, M.C., Bryant, H., Beck, M.R., Fleming, A.E., Gregorini, P. (2020). Grazed chicory, plantain or ryegrass–white clover alters milk yield and fatty acid composition of late-lactating dairy cows. *Animal Production Science* 60; 107–113.

Mangwe, M.C., Bryant, H. Gregorini, P. (2020). Rumen fermentation and fatty acid composition of milk of mid lactating dairy cows grazing chicory and ryegrass. *Animals* 10; 169.

Mangwe, M.C., Bryant, R.H., Moreno García, C.A., Maxwell, T.M.R., Gregorini, P. (2020). Functional Traits, Morphology and Herbage Production of Vernalised and Non-Vernalised Chicory cv. Choice (*Cichorium intybus* L.) in Response to Defoliation Frequency and Height. *Plants* 9; 611.

Accepted journal articles

Mangwe, M.C., Bryant, R.H., Gregorini, P. Effect of vernalisation on the diurnal changes in fatty acid profile and nutrient composition of chicory in response to defoliation frequency. *New Zealand Journal of Agricultural Resea**rch*****;** Accepted pending minor revisions.

Manuscripts under preparation

Mangwe, M.C., Bryant, R.H., Gregorini, P. Differing response in urination behaviour from including chicory forage into the ryegrass-white clover diet of dairy cows.

Peer reviewed international conference abstracts and presentations

Mangwe, **M.C.,** Bryant, R.H., Gregorini, P. (2018). Grazed chicory, plantain, or ryegrass-white clover alters milk yield and fatty acid composition of late lactating dairy cows. *Australasian Dairy Science Symposium*. Palmerston North, New Zealand.

Mangwe, **M.C.,** Bryant, R.H., Beck, M.R., Gregorini, P. (2019). Milk yield and urine excretion patterns of dairy cows grazing second-year chicory-based herbage. 70th Annual Meeting of the European Federation of Animal Science in Ghent, Belgium August. pp. 557.

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Mancoba M.C., Bryant R.H. (2020). CHICORY'S PROMISE – BETTER MILK, LESS URINARY N. *Green to Gold* 2020, Issue 198.

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List of Abbreviations

Abbreviation	Description
ADF	Acid detergent fibre
ALA	α-linolenic acid
CH	Chicory
CHAM	RGWC + morning allocation of chicory
CHPM	RGWC + afternoon allocation of chicory
CLA	Conjugated linoleic acid
CP	Crude protein
CT	Condensed tannins
DM	Dry matter
DMD	Dry matter digestibility
DOMD	Digestible organic matter in the dry matter
FA	Fatty acid
FWI	Feed water intake
GDD	Growing degree-days
K	Potassium
LA	Linoleic acid
ME	Metabolizable energy
MUN	Milk urea nitrogen
N	Nitrogen
NA	Sodium
NDF	Neutral detergent fibre
NFC	Non-fibre carbohydrates
NIRS	Near infrared spectrophotometry
NUE	N use efficiency
OM	Organic matter
OMD	Organic matter digestibility
PCA	Principal component analysis
PR	Perennial ryegrass
PUFA	Polyunsaturated FA
RGWC	Perennial ryegrass/white clover
SEM	Standard error of the mean
SFA	Saturated FA
SL	Sesquiterpene lactones
SLA	Specific leaf area
SPAD	Soil Plant Analysis Development
TWI	Total water intake
UN	Urinary nitrogen
VA	Vaccenic acid
VFA	Volatile fatty acids
WSC	Water soluble carbohydrates

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Chapter 1
1. Introduction

1.1 Introduction

The dairy industry in New Zealand is an important contributor to the economy of the country, contributing nearly 3.5% to the country's total gross domestic products (Ballingall and Pambudi, 2017). The dairy industry in New Zealand is pasture based with perennial ryegrass/white clover (*Lolium perenne* L./*Trifolium repens* L.; **RGWC**) mix as the main pasture-species used to support the high producing animals. The reliance on this sward mix relates to its high nutritive value (Waghorn et al., 2007), ease of establishment and tolerance to a wide range of grazing regimes (Kemp et al., 2000). In recent decades, grazed livestock production systems in temperate regions have focussed on increasing productivity in order to meet the growing global demand of ruminant-source foods. This increase in farm productivity has been achieved through improved animal genetics, increased use of fertiliser and irrigation and maximizing herbage production of the traditional pasture species. However, this has resulted in negative environmental impacts, particularly around fresh water quality, challenging the sustainability of grazed systems (Beukes et al., 2012). In future, the use of water and fertilisers may be reversed so the role of alternative forages is being considered in facing these issues.

One of the key aspects of the environmental challenge associated with RGWC is the high crude protein (**CP**) concentration in the herbage which frequently exceeds nutritional requirements of high producing dairy cows [Figure 1.1; (Bryant et al., 2019)]. When consumed, the high protein diet results in over sixty percentage of the consumed N being excreted in the urine and faeces, thereby contaminating the environment (Kebreab et al., 2001). Urine is one of the major sources of N degrading the natural water bodies nearby agricultural activity (Wachendorf et al., 2005). This is mainly because the N deposited in urine patches [200 to 2000 kg/ha N (Selbie et al., 2015)] is often at a rate too high for plants to use, resulting in accumulation of nitrate in the soil and ultimately N loss through leaching when drainage occurs (Scholefield et al., 1993; Wachendorf et al., 2005). Consequently, strategies are being explored to attenuate N losses to achieve regulatory limits (Bryant et al., 2019).

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Figure 1.1. Variation in the dietary crude protein surplus of upper North Island and South Island perennial ryegrass/white clover-based swards. The solid line shows the average crude protein (% of DM) content of swards while the dashed line represents the minimum crude protein content (% of DM) required by a dairy cow in the upper North Island producing 340 kg milk solids per year or in the South Island producing 400 kg milk solids per year. Sourced from Bryant et al. (2019).

Several studies have highlighted the environmental benefits from alternative forages based on a range of plant traits including winter activity for reduced nitrate leaching (Malcolm et al., 2018); high moisture content to reduce urinary N load (Bryant et al., 2019), and increased soil water use to reduce drainage (Brown et al., 2003; Welten et al., 2019). Chicory (*Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.) possess many of the attributes required to improve pastoral farm systems. The beneficial effects of including plantain to reduce the environmental impacts associated with livestock production have been explored extensively, as reviewed by Bryant et al. (2019). As an example, including plantain in the RGWC diets has demonstrated potential to reduce the concentration of urinary nitrogen (**UN**), which may diminish N loss from pastures without negating milk production (Box et al., 2017a; Bryant et al., 2017; Woods et al., 2018). Chicory is another forage herb that has the attributes required to reduce the concentration of UN. For example, chicory has a higher moisture content than RGWC; a trait that has been identified as a means to reduce UN load and subsequent nitrate leaching from dairy cattle grazing in pastoral systems (Bryant et al., 2019). This thesis explores the potential use of chicory as an alternative to the traditional RGWC to reduce the environmental impacts associated with pastoral systems in New Zealand.

As consumer awareness of food choices increases with respect to health choices, animal welfare and environmental impact of food, the milk quality from pastoral grazed systems is becoming important. This is driven mainly by the elevated concentration of health promoting fatty acids (**FA**) in ruminant products from grazed systems compared with those from concentrates or conserved feed-based systems (Elgersma, 2015). Specifically, nutritionists have indicated interest in milk FA profile as some individual FA have implications on human health. Polyunsaturated FA (**PUFA**) have been associated with many human-health related benefits (Palmquist, 2009). While, milk saturated FA (**SFA**) have been linked to obesity, diabetes, cardiovascular disease and cancer in humans (Lavie et al., 2009; Shingfield et al., 2013). Therefore, an increased intake of conjugated linoleic acid (**CLA;** isomer C18:2 c9 t11) and Omega-3 FA and a decrease in SFA and Omega-6 from milk and milk product to help curb the occurrence of cardiovascular and other chronic conditions is recommended (Toral et al., 2018). The FA profile of ruminant-source foods is to a larger extent, determined by the diet of animal (Dewhurst et al., 2006). Increased concentration of PUFA in the herbage has been linked with increased concentration of the functional FA in the milk (Jenkins et al., 2008; Toral et al., 2018). Therefore, strategies to enhance the levels of PUFA in the of diet of ruminants in order to substantially improve the milk FA quality are being considered.

At the farm level, it is possible to enhance the concentrations of functional FA in milk through supplementation with novel forage herbs with high PUFA concentrations (Lourenço et al., 2008). Alternative forages such as chicory have higher concentrations of PUFA in the herbage as compared with perennial ryegrass [**PR** (Muir et al. 2014, 2015)]. A few studies show that chicory feeding can improve the concentration of Omega-3 and CLA in meat and milk of ruminants (Muir et al., 2015; Rodríguez et al., 2020). However, to capture the value of chicory as a means for improving product quality in terms of milk FA, more information is required to understand the mechanisms leading to increased PUFA from chicory diets and the associated feeding management.

The adoption of chicory on farm requires confidence in the response to management decisions and needs to identify risks to production in different environments. Concerns over the development of the reproductive stem in winter vernalised chicory plants and its limited persistence have slowed the adoption of the herb in pastoral systems (Li and Kemp, 2005; Lee et al., 2015a). Reproductive stems of vernalised chicory plants compromise the feeding value of the herbage. While several other studies have investigated management options to control the growth and development of reproductive stems in chicory plants (Clark et al., 1990a; Li et al., 1994; Lee et al., 2015a), the mechanisms and factors that trigger and influence the development and elongation of reproductive stems in vernalised chicory are less well defined and need further research. In particular, empirical studies investigating the leaf and stem development dynamics of vernalised chicory in response to varying defoliation intensity and regrowth interval are important to generate efficient and specific defoliation strategies of chicory pastures that could be used to control production and feeding value of chicory on farm.

Studies suggest that chicory persist for 3 and 7 years dependent on suitability of climate, establishment and defoliation management (Hume et al., 1995; Li et al., 1997a). One key limitation of chicory is the poor survival of the new seedlings recruited in autumn or spring (Volesky, 1996). Therefore, it is important to maintain an adequate plant population of the original plants of chicory in the sward as a decline in plant populations can affect herbage production. While several studies have investigated management options to enhance the persistence of the herb (Clark et al. 1990; Li et al. 1997; Labreveux et al. 2004; Lee et al. 2015), the criterion used to determine when to defoliate chicory in all these studies was based on calendar days or sward surface height. Fixed number of calendar days does not consider climatic variation between seasons and locations that will affect both the stage of regrowth and amount of herbage present at the end of each specified period (Fulkerson and Donaghy, 2001). The use of morphological traits as a criterion of when to defoliate forages in grazed systems has been suggested as a more specific and generic alternative defoliation interval creterion (Fulkerson and Donaghy, 2001). Morphological, physiological and functional traits have been useful to characterize plant species regarding their strategies to acquire, store, and invest nutrients and energy as well as to respond to variable frequency and height of defoliation (Briske and Richards, 1995; Gastal and Lemaire, 2015). Therefore, there is need for empirical studies on mophological, functional and physiological characteristics of chicory in relation to thermal time and management regime.

It is also important that we consider the nutritive value and FA concentration of the herbage when designing optimum grazing management regimes of chicory. Crude protein and metabolizable energy (**ME**) for example, are the key drivers of milk production, milk composition and body weight. Grazing management practices including defoliation interval and severity have been identified as major factors influencing the nutritive components of forages in pastoral systems (Briske and Richards, 1995). Furthermore, the composition of FA in the herbage is driven as much by forage type as by management practices (Dewhurst et al., 2006; Lourenço et al., 2008). Information available in the literature show that the levels of PUFA in PR herbage varies considerably, depending on growth stage, management practices such as defoliation interval (Dewhurst et al. 2001; Elgersma et al. 2003a). Growth stage and defoliation interval affect the morphology of the crop and therefore, the herbage FA composition (Dewhurst et al., 2001, 2003; Elgersma et al., 2003a). While these changes are well documented for PR, studies aiming at quantifying and describing such trends in chicory forage are lacking.

1.2 Aim of the thesis

The main aim of the thesis is to investigate the effects chicory-based herbage has on urine N excretion, milk production and milk FA composition of dairy cows, as well as to understand the mechanisms leading to the variation in milk FA of cows grazing chicory-based herbage and those grazing RGWC-based herbage. A secondary aim is to investigate the effect of grazing management (defoliation intensity, severity and timing) before and after vernalisation on morphology, functional traits, herbage DM production and biochemical composition of chicory and the subsequent effect on milk production and milk FA composition. The specific aims were:

a. To compare the effect of grazed chicory-based herbage to that of the traditional RGWC-based herbage on milk production and milk FA composition.
b. To compare the effect of grazed chicory-based herbage to that of the traditional RGWC-based herbage on urination behaviour and nitrogen partitioning.
c. To investigate the effect of defoliation interval, severity and time of day on agronomic and chemical response of chicory.

1.3 Thesis structure

A diagrammatic representation of this thesis structure is given below. This thesis is presented in nine Chapters with Chapter 1 being an introduction. Chapter 2 is a literature review, outlining the agronomic features of chicory under various defoliation management strategies and N fertiliser application rates across different seasons. The review further examines the effects of feeding chicory on milk production, FA composition and urine nitrogen N excretion of dairy cows. Chapters 3 and 4 represent the first grazing experiment (a baseline study), which compared milk production, milk FA composition, urination patterns and N use efficiency of cows grazing chicory, plantain (control herb), or ryegrass/white clover herbage (control grass). Chapters 5 and 6 represent the agronomy experiments of the thesis, which examined the effects of defoliation regimes on functional traits, morphology, herbage production, nutrient composition and FA composition of chicory, before and after vernalization. Chapters 7 and 8 represent the final grazing experiment. The treatments used in the second grazing experiments were based on the results obtained from the previous experiments (Chapters 3 – 6). The aim of second grazing study was to investigate the effects of including chicory into the traditional feeding regime of ryegrass/white clover, and time of its allocation on milk production, rumen fermentation, FA composition, urination patterns and N use efficiency. Chapter 9 draws all the results together, with the overall conclusion presented at the end of the Chapter.

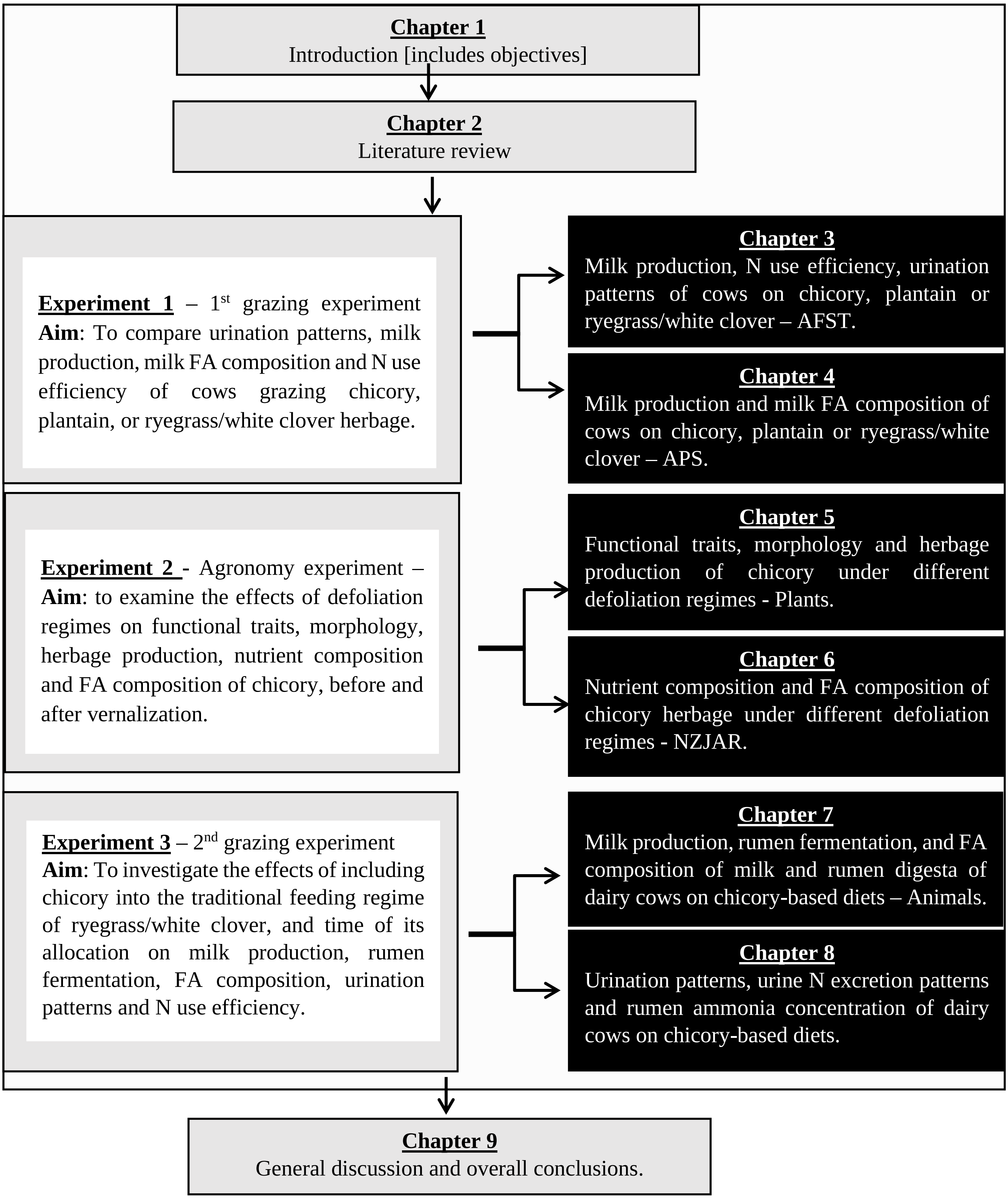

Figure 1.2. A diagrammatic representation of the structure of the thesis

Chapter 2

2. Literature Review

2.1 Introduction

This review of literature will outline the agronomic features (i.e. morphological traits, chemical composition, FA profile and herbage production) of chicory under various defoliation management strategies and N fertiliser application rates across different seasons. Where available, comparisons will be made between chicory and PR. The review will further examine the effects of feeding chicory on milk production, FA composition and urine nitrogen N excretion of dairy cows.

2.2 Agronomic features of chicory

2.2.1 Background information

Chicory is a bioactive forage herb that belongs to the Asteraceae Family. It is native to Europe and Asia but is also found in other parts of Africa and America (Clapham et al., 1962). The herb has, for a long time, been used as a forage crop in temperate regions. Though earlier studies wrote that the herb was traditionally considered a weed as it was found in natural grasslands and roadsides (Rumball, 1986). Foster (1988) reported that evaluations of chicory as a forage crop began in the early 1900s in the United Kingdom. While chicory was considered a weed species in the USA until Jung et al. (1996) conducted a series of experiments during the year 1993 to allow for its usage as a forage crop. In New Zealand, the use of chicory as a forage crop began in the early-1900s where it was often included in grass seed mixtures (Rumball, 1986). The herb had the attributes required of a forage species to grow well on low fertility soil that dried out in summer (O'Brien, 1955). However, its poor persistence and the development of the seed heads in winter vernalised chicory plants limited its usage a forage crop (O'Brien, 1955). Much later, chicory was re-evaluated as a pasture species in New Zealand and gained popularity in the mid-1970s. The first commercial cultivar of chicory ''Grasslands Puna'' was therefore released in New Zealand in 1985 following 10 years of breeding (Rumball, 1986). The use of chicory as commercial forage crop has now spread worldwide (Li and Kemp, 2005; Peña-Espinoza et al., 2018), and several forage chicory cultivars have been developed.

There are two main cultivars of chicory currently used in pastoral systems in New Zealand; Grassland Puna II and Grassland Choice (Glassey et al., 2013; Lee et al., 2015a). Both cultivars were bred entirely from Grassland Puna. Grassland Puna II was bred to grow well in cool seasons, for greater concentrations of the sesquiterpene lactones (**SL**), greater uniformity and for tolerance to the fungus *Sclerotinia* (Rumball et al., 2003c). The cultivar was envisioned for use on non-milking platforms as part of diverse swards or as a monoculture. Grassland Choice was bred to grow well in winter, for greater morphological uniformity and for lower concentrations of the SL. It was envisioned for use on dairy farms, where the lower concentrations of SL are less likely to cause milk taint (Rumball et al., 2003a).

Since the early 1990s, a plethora of mitigation strategies to improve the persistence of chicory without compromising its nutritive value have been evaluated (Li et al., 1997a; Clapham et al., 2001; Lee et al., 2015a). Grazing management practices including defoliation interval and severity have been identified as major factors influencing these aforementioned parameters in pastoral grazed systems (Li and Kemp, 2005). However, studies investigating grazing interval for chicory have used calendar days or sward surface height as a criterion of when to defoliate the herb. Fulkerson and Donaghy (2001) has challenged this idea as fixed number of calendar days does not consider climatic variation between seasons and locations that will affect both the stage of regrowth and amount of herbage present at the end of each specified period. Additionally, most studies were conducted in rainfed environments without irrigation. In natural rain fed environments, where summer moisture deficits are common, chicory has shown to be more sensitive to defoliation interval than defoliation height (Labreveux et al., 2004; Li and Kemp, 2005; Lee et al., 2015a). The partitioning of photosynthates to above ground biomass of chicory is reduced under moisture deficit (Monti et al., 2006; Cranston et al., 2016), so morphological response of chicory (to defoliation interval and/or height) may different an irrigated environment.

The use of leaf stage as a criterion of when to defoliate forages in grazed systems has been suggested as a more specific and generic alternative (Fulkerson and Donaghy, 2001). However, the avialable information on the utility of leaf growth stage as a determinant of defoliation interval is limited to perennial grasses. It is imperative to conduct such studies on the novel forage herbs such as chicory inorder to improve the efficancy of their grazing management at farm level. This requires information on leaves, stems and roots development dynamics of the plant at different growth stages and across seasons in order to properly predict herbage production and nutritive value of the forage under various management regimes (Clapham et al., 2001). Hence, the need for empirical studies on mophological, functional, physiological and biochemical characteristics of chicory in relation to thermal time and management regime.

2.2.2 Morphology and functional traits of chicory

2.2.2.1 Below ground

Chicory is described as a deep-rooted forage herb that is more drought tolerant than the traditional PR (Li and Kemp, 2005) and plantain (Lee et al., 2015a; Cranston et al., 2016). The deep-rooting system of chicory enables the herb to access water as deep as 1.9 m in the soil profile (Brown et al., 2005). Dowling et al. (2006) wrote that chicory can grow up to 38 kg herbage per ha per day per mm of rainfall. Chicory was found to maintain a higher green-leafiness than grasses and legumes during dry conditions in Australia and New Zealand (Nie et al., 2008)

Chicory stores most of its carbohydrate reserves in the taproots (Lee et al., 2015b). Several studies have linked chicory plants survival during winter and its recovery during spring to the reserves stored in the roots (Li et al., 1997a; Lee et al., 2015a; b). Therefore, the amount of carbohydrates stored in the roots before winter is important for the survival of the herb in the subsequent seasons. The concentration of the reserves in grazed herbaceous plants is influenced by both defoliation interval and severity (Solomon et al., 2017). As an example, Donaghy and Fulkerson (1997) found that PR defoliated after shorter intervals (before the two leaf stage) had reduced concentration of reserves in roots than those defoliated after longer intervals (three to four leaf stages), which affected the growth rate of the plants. Perennial ryegrass tends to accumulate reserves until the plant has four fully developed leaves, therefore defoliation before then compromises the longevity of the plants (Fulkerson and Slack, 1994). In the case of chicory, stronger evidence of the beneficial effects of longer intervals on non-fibre carbohydrates (**NFC**) reserves in the roots was provided by a study that used extended leaf height as a criterion of when to defoliate chicory swards (Lee et al., 2015a; b). Lee et al. (2015b) studied the patterns and time required to rejuvenate NFC reserves for chicory roots during a 35-day regrowth cycle in summer. The authors observed a decline in root NFC for the first seven days after defoliation before replenishment began. Pre-defoliation levels were attained by day twenty-one of regrowth, which was equivalent to 310 growing degree-days (**GDD**, assuming a base temperature of 5°C). However, the impacts of defoliating before or after full replenishment of the reserves on growth rate and herbage production of chicory have not been studied. Additionally, there is no information on the seasonal carbohydrates reserves dynamics of the roots of chicory plants under different management regimes, information that is required to predict and balance management of the crop to ensure stable herbage production and persistence.

2.2.2.2 Above ground

The morphology of perennial herbaceous plants determines herbage production and nutritive value of the herbage, with greater stem proportion resulting in increased herbage mass but decreased forage quality (Chen et al., 2019; Ta et al., 2020). Therefore, the main goal in chicory grazing management is to maximize leaf production and minimize stem production (Matthews et al., 1990; Hume et al., 1995; Li et al., 1997c). This requires an understanding of the development dynamics of the aerial plant parts in relation to thermal time to establish optimal and efficient grazing management strategies of chicory pastures that could be used to control herbage production and nutritive value of the herb.

Studies on chicory have revealed that the morphology of chicory varies between phenological stages (Li et al., 1997b; Clapham et al., 2001). After establishment and before vernalisation in winter, chicory plants remain vegetative with one shoot per plant. The shoot composes of a whorl of 5 to 15 leaves, with average mature size ranging from 40 to 60 cm^2 (Clapham et al., 2001). Vernalisation of chicory is triggered at low temperatures between 0 and 12 °C (Cichota et al., 2020). Gianquinto (1997) and Dielen et al. (2005) reported that 10 vernalising degrees-days are required to trigger the end of vegetative phase of chicory. In temperate regions, this occurs in winter. When the plant is vernalised, the initial rosette of chicory divide into multi-crowns (Hare et al., 1987; Li et al., 1997c). A primary stem shoots from the main rosette, while lateral stems sprout from the primary stems. The reproductive stems have many small leaves averaging 10 cm^2 (Clapham et al., 2001). The primary stems can grow up to two metres when left uncontrolled (Rumball, 1986). As the stems of perennial herbaceous plants become longer and matured, the nutritive value of the forage is compromised due to a lowered leaf-to-stem ratio. The development of the reproductive stem in winter vernalised chicory plants has slowed its adoption in pastoral systems. Therefore, management strategies designed to control the growth and development of reproductive stems in vernalised chicory plants are strongly desired.

Li et al. (1997c) compared the morphology of individual plants of chicory under different defoliation frequencies. In the plants that were defoliated every four weeks, individual plant size was significantly greater than those defoliated every one or two weeks due to increases in stem mass. Several other studies have also investigated management strategies to control the growth of stems and therefore the feeding value of the herbage (Clark et al., 1990a; Labreveux et al., 2006; Lee et al., 2015a). The authors concluded that longer regrowth intervals after vernalisation decreased the feeding value of chicory due to the increased stem material in the herbage. In a review, Li and Kemp (2005) recommended that grazing management strategies should be designed to maintain a desirable 30:70 stem to leaf ratio. This requires an understanding of the mechanisms and factors that trigger and influence the development and elongation of reproductive stems in vernalised chicory. Clapham et al. (2001) investigated the development dynamics of vernalised chicory cv. Puna in relation to thermal time and reported that time to bolting was 400 growing degree-days (GDD). Little is known about the development dynamics (i.e., bolting initiation and stem elongation) of the commercially available chicory cv. Choice. If the objective is to control the growth and development of the reproductive mature stems, chicory should be grazed before bolting to maintain plants in the vegetative state (Barry, 1998a). Understanding the morphological and physiological response to defoliation before bolting, i.e., 300 GDD or after bolting, i.e., 600 GDD and the traits associated with this is therefore strongly desirable to generate efficient and specific defoliation strategies of chicory pastures that could be used to control production and feeding value of chicory on farm.

2.2.3 Herbage production and pattern of growth

2.2.3.1 Herbage production

The annual herbage mass of pure swards of chicory varies considerably, depending on environmental conditions and management regimes. Tables 2.1-2.3 summarises the main findings from several peer-reviewed studies. Under optimal conditions, herbage production of ≥19.0 t DM/ha/year have been reported in Canterbury, NZ (Brown and Moot 2004) and New South Wales, Australia (Neal et al., 2009). Compared with the traditional PR, chicory sown in mixtures with grass and clover or sown as a monoculture can produce similar or greater herbage yield, particularly during warmer seasons (Neal et al., 2009; Nobilly et al., 2013).

Generally, herbage production of pure swards of chicory decreases as the crop ages (Table 1). Jung et al. (1996) in Pennsylvania, USA compared herbage mass of Puna chicory defoliated at 25, 37 or 50 cm canopy height over a two-year period. An average of 16% decrease in herbage mass in year two compared with year one was reported across defoliation frequencies. In a four-year study conducted in Palmerston North, New Zealand, a two-fold reduction in herbage mass in year four compared with years one and two was also observed for chicory swards, which was directly related to the decline in plant density [Table 2.1; (Li et al., 1997a)].

Table 2.1. Annual herbage mass (tonnes DM/ha) of pure swards of chicory across several studies.

Study	Region	Year	Annual yield
Jung et al., 1996	Pennsylvania, USA	Year 1	9.4
		Year 2	7.9
Volesky, 1996	Oklahoma, USA	Year 1	7.5
		Year 2	7.7
Li et al., 1997a	Palmerston North, NZ	Year 1	8.5
	Nov - April	Year 2	9.4
		Year 4	4.6
Belesky et al., 1999	West Virginia, USA	Year 1	7.8
		Year 2	6.3
		Year 3	7.6
Brown and Moot, 2004	Canterbury, NZ	Year 1	19.0
		Year 2	17.1
		Year 3	15.2
Neal et al., 2009	New South Wales	Year 1	19.8
	Australia	Year 2	14.6
		Year 3	15.0
Lee et al., 2015a	Hamilton, NZ	Year 1	10.6
Dec - May		Year 2	6.5

The effects of various management strategies such as defoliation management, irrigation and N fertiliser rates to enhance herbage mass of pure swards chicory are presented in Tables 2.2-2.3. The effect of longer defoliation interval of the first commercial cultivar, Puna chicory, was first suggested by Clark et al. (1990a) in a study conducted from 26 October to 21 December 1988 in Palmerston North, NZ, describing chicory exposed to various grazing intervals. Pure swards of chicory defoliated every-one, two, four or eight weeks produced 2187 kg/ha, 3262 kg/ha, 4869 kg/ha, and 6402 kg/ha DM of herbage mass, respectively. Subsequent studies in New Zealand were in agreement with these findings (Table 2.2); e.g. Li et al. (1997c) found 71% greater herbage mass in chicory swards defoliated every four weeks in comparison with swards defoliated every one or two weeks. The observed increase in herbage produced under longer defoliation intervals has been attributed to the greater stem proportion when compared with shorter defoliation intervals (Clark et al., 1990a; Li et al., 1997a; Lee et al., 2015a).

Table 2.2. Effect of defoliation interval on herbage mass (tonnes DM/ha) of pure chicory swards across several studies.

Study	Region	Interval	Spring	Summer	Autumn	Total
Clark et al., 1990	Palmerston North, NZ	1 week				2.2
	Oct - Dec	2 weeks				3.3
		4 weeks				4.9
		8 weeks				6.4
Jung et al., 1996	Pennsylvania, USA	25 cm				8.2
		37 cm				8.4
		50 cm				11.0
Li et al., 1997b	Palmerston North, NZ	1 week				4.9
	Nov 1994 – April 1995	2 weeks				6.4
		4 weeks				9.6
Lee et al., 2015a	Hamilton, NZ	250 cm	4	6	2.1	12.1
		350 cm	4.8	6.7	2.2	13.7
		450 cm	6.2	6.3	2.05	14.6
Lebreveux et al., 2004	Pennsylvania, USA	1998	6.5	3.3		9.8
		2000	5.4	3.2		8.6
		2001	5.7	3.5		9.2

Chicory needs an external N supply to enhance herbage production (Clark et al., 1990a; Collins and McCoy, 1997; Belesky et al., 2000; Martin et al., 2017a). In the study reported by Clark et al. (1990a), in which chicory swards received increasing N fertiliser rates, herbage yields of 3029 kg DM/ha, 3910 kg DM/ha, and 5684 kg DM/ha for N rates of 0 kg N/ha, 50 kg N/ha, and 200 kg N/ha, respectively were reported (Table 2.3). Collins and McCoy (1997) found linear increases in herbage mass of chicory during the first year and quadratic increases during the second year with increases from 0 to 200 kg N/ha fertiliser rates. In a recent cut and carry study, chicory herbage production increased linearly from approx. 8000 kg DM/ha per year to 12000 DM/ha per year with an increase from 0 to 450 kg N/ha fertiliser rates per year (Martin et al., 2017). However, increasing fertiliser rates up to 450 kg/ha can be detrimental to the environment. Li and Kemp (2005) suggested fertiliser rates between 20–50 kg N/ha in early spring to be enough to assist the chicory plants' recovery from winter. In addition, other studies have reported negative effects of N fertiliser rates on plant density of chicory swards which might affect herbage production in the long run (Clark et al., 1990a; Belesky et al., 2000). For example, in a study in West Virginia, in which chicory was defoliated every six weeks for three years, increasing N fertiliser rates from 0 to 480 kg N/ha reduced plant density from nearly 40% (0 N) to 5% (480 N) of the initial plant population after three years (Belesky et al., 2000). Similarly, Collins and McCoy (1997) reported that chicory plants averaged 67 plants/m^2 when no N fertilisation was applied compared with 35 plants/m^2 at the 150 kg N/ha at the end of a three year experiment.

Table 2.3. Effect of nitrogen fertiliser rates on herbage mass (tonnes DM/ha) of pure chicory swards across several studies.

Study	Region	N Rate	Yield
Clark et al., 1990	Palmerston North, NZ	0 kg N/ha	3.0
	Oct - Dec	50 kg N/ha	3.9
		200 kg N/ha	5.7
Belesky et al., 2000	West Virginia, USA	0 kg N/ha	3.5
Year 1		50 kg N/ha	3.7
		200 kg N/ha	4.6
		480 kg N/ha	6.2
Martin et al., 2017	Lincoln, NZ	0 kg N/ha	8.0
		50 kg N/ha	8.4
		200 kg N/ha	9.8
		450 kg N/ha	12.1

2.2.3.2 Growth patterns

Chicory is very responsive to changes in temperature, being most active during the high temperatures in spring and summer, and practically inactive during the cold temperatures in winter (Jung et al., 1996). Brown et al. (2005) observed growth rates of up to 90 kg DM/ha per day in January, 50 kg DM/ha per day in February and practically no growth in winter Canterbury, NZ. Growth rates above 150 kg DM per/ha per day have been reported in other parts of New Zealand in summer (Hare et al., 1987; Matthews et al., 1990).

By contrast, the productivity and quality of the traditional RGWC is low during the high temperatures in summer relative to spring [Figure 2.1; (Bryant et al., 2009)], which often limits livestock production. Neal et al. (2009) compared the productivity of various forages at Camden, NSW, Australia under optimum irrigation and selected deficit irrigation (66% or 33% of irrigation water applied). The experiment ran for three years and the advantages of chicory over PR in summer were seen, with decreases in herbage production from RGWC swards exacerbated under arid conditions (Neal et al., 2009). For example, during the second year, chicory swards produced 3.1, 4.2 and 7.6 t DM/ha under 33%, 66% and 100% irrigation in summer, which was greater than the 1, 2, and 6 t DM/ha for PR (Neal et al., 2009). The superior performance of chicory during summer and under arid conditions compared with PR has been accredited to its considerable summer heat tolerance and the capacity of the deep roots to extract water from deeper soil levels (Brown et al., 2003; Li and Kemp, 2005). This growth pattern of chicory is particularly important in the pastoral system as chicory may enhance sward productivity and feed quality over the summer, when PR swards often limits livestock production (Li and Kemp, 2005; Nobilly et al., 2013). For example, dairy cows in New Zealand calve early spring and are at mid lactation over summer. As depicted in Figure 2.2, the demand for high quantity and quality feed for dairy cows is high in early spring during calving and remains high over summer during mid lactation, and the seasonal growth pattern of chicory fits the requirements of dairy cows for high feed quantity and quality in late spring and summer.

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Figure 2.1. Pasture growth rate of three perennial ryegrass cultivars. Sourced from Bryant et al. (2009).

Material removed for copyright compliance

Figure 2.2. Pasture growth and animal feed requirements for dairy farms in New Zealand. Sourced from Holmes et al. (2002).

2.2.4 Persistence

Whilst chicory has demonstrated potential benefits to the pastoral system through increased herbage production during dry seasons when the productivity of the traditional RGWC is low, the persistence of the herb is limited (Hume et al., 1995; Li et al., 1997a). In a review, it was reported that chicory persist for 3 – 7 years, depending on suitability of climate, establishment and defoliation management (Li and Kemp, 2005). One key limitation of chicory is the poor survival of the new seedlings recruited in autumn or spring (Volesky, 1996). Therefore, it is critical to maintain an adequate plant population of the original plants of chicory in the sward as a decline in plant populations can affect herbage production. However, managing chicory to maintain plant populations has proven to be challenging.

In an 18-month study, in which chicory was subjected to several defoliation frequencies and intensities, the plant population of chicory swards was 41% of the initial plant numbers after 18 months (Lee et al., 2015a). Defoliation frequency and intensity had minor effects on plant populations. A decline of 31% in plant populations every year is not uncommon in chicory swards (Volesky, 1996; Li et al., 1997a). Clapham et al. (2001) also observed a 33% decline of the plants between 1997 and 1998 growing seasons. Volesky (1996) found that a population of 43–48 plants/m^2 seemed to be the maximum for swards from the second growing season.

Li et al. (1997c) revealed that as plant population declined in a stand, they tend to increase their individual size. For example, the number of shoots per plant increased from three shoots/plant during the first year to seven shoots/plant during the fourth year (Li et al., 1997c). The increase in plant size was found to compensate for the decline in plant population, thus maintaining herbage production. However, the unavoidable decline in plant population subsequently decrease yields per unit area in the long run. Under New Zealand conditions, Li et al. (1997c) found that once the plant population of chicory declined below 25 plants/m^2, herbage production was likely to be fifty percent or less of its maximum. Li and Kemp (2005) recommended that farmers would have to re-establish chicory in this state. Therefore, there is need to generate grazing management strategies that could enhance the persistence of the herb.

2.2.5 Nutritive value

2.2.5.1 Organic compounds

2.2.5.1.1 Crude protein

The CP requirements of dairy cows vary between 14 to 18% of dry matter (**DM**), depending on stage of lactation (NRC 2001). Generally, the CP content of forages used in pastoral dairying systems located in temperate regions exceed these requirements. Although rare, the traditional RGWC forage under low N fertiliser rates can have an average CP concentration of 119 g CP/kg DM in dry summer conditions (Box et al., 2017b), which is below the CP requirement for a high producing dairy cow. Under these conditions, the use of alternative forages with high CP content can be beneficial.

Table 2.4 summarises the CP concentration of chicory compared with plantain and/or PR. The average CP concentration of chicory across the reported studies was 20.2%, which was greater than the 18.1% and 17.7% for plantain and PR, respectively. However, the CP concentration of chicory varied substantially depending on phenological stage. Generally, non-vernalised chicory contained greater CP concentration than vernalised chicory, due to the higher leaf-to-stem ratio in non-vernalised than vernalised chicory herbage (Lee et al., 2015a). Chicory leaf material has higher CP concentration than stem material (Table 2.4; Brown et al., 2003; Li et al., 1997b).

The CP concentration of chicory also vary between defoliation intervals due to differences in the morphology of the plants. The 23.1% CP from chicory swards defoliated every 25 cm canopy height was significantly greater than the 13.8% CP from chicory swards defoliated every 50 cm canopy height in Pennsylvania, USA (Jung et al., 1996). Likewise, the 26.3% CP from chicory plants defoliated at 250 cm extended leaf height was significantly greater than the 20.6% and 19.4% CP from chicory swards defoliated at 350 and 450 cm extended leaf height in Hamilton, New Zealand (Lee et al., 2015a). Greater CP concentrations were observed in chicory swards that received 450 kg/ha N fertiliser than those that received 0 or 180 kg/ha N fertiliser [Table 2.2; (Box et al., 2017c; Martin et al., 2017b)].

2.2.5.1.2 Neutral detergent fibre

Forage digestibility is related to the fibre content of the forage, with high neutral detergent fibre (**NDF**) concentrations associated with lower digestibility coefficients. The fibre content of chicory is lower than that of RGWC herbage (Table 2.4), indicating that chicory is more digestible than the traditional PR herbage. Moreover, the fibre content of chicory is less than the recommended 30-40% for lactating dairy cows diets to maintain optimum rumen functioning (NRC, 2001). Waugh et al. (1998) suggested that feeding large amounts of chicory may result in milk fat depression due to this low NDF concentration. Several researchers have offered chicory with other forages to overcome the low NDF concentration encountered in pure swards of chicory (Minneé et al., 2012, 2017; Muir et al., 2014, 2015).

2.2.5.1.3 Non-fibre carbohydrates

Greater concentrations of NFC in chicory than PR herbage (14.2 vs 7.7% of DM) were found when it was offered to dairy cows during an indoor experiment in summer in Hamilton, New Zealand. Kusmartono (1996a) also found that the ratio of NFC to fibre carbohydrate was nearly three times greater in chicory than PR herbage grazed by deer in Palmerston North. The levels of NFC of herbaceous forages vary during the day (Delagarde et al., 2000a). Box et al. (2017b) found greater water-soluble carbohydrates concentrations and lower CP and NDF concentrations in chicory herbage harvested during the afternoon than during the morning. However, the time of day effect was greater on RGWC herbage than chicory herbage (Box et al., 2017c). It would also be interesting to investigate the effects of the diurnal changes in nutritive composition of the traditional RGWC and chicory herbages and their interactions on ingestion, rumen fermentation, milk production, milk fatty acid composition and urinary **N** losses in grazed pastoral systems as a means to evaluate the feasibility of including the herb to a traditional PR diet of dairy cows. Several authors have used the diurnal changes in nutritive value of PR to create an increase in the concentration of NFC at the expense of CP in order to enhance the utilisation efficiency of the herbage CP (Miller et al., 2001; Moorby et al., 2006).

Increases in NFC at the expense of CP and the fibre concentration of the herbage is strongly desirable to grazing livestock to enhance production while minimizing N loss via urea (Edwards et al., 2007). While this is well documented for PR, there is no information regarding the effect of the diurnal fluctuations of nutrient components of chicory on dairy cows' milk production N use efficiency and therefore N loss.

2.2.5.1.4 Metabolizable energy

Crude protein and metabolizable energy (**ME**) are the key drivers of milk production and body weight gain. However, in temperate regions, it would be rare for cows to be deficient in CP. Therefore, DM intake of dairy cows in temperate regions are more strongly influenced by ME rather than CP concentration of the diet. The main feed energy sources are soluble carbohydrates (starch, sugar) and fibre. The ME concentration of the traditional PR varies between seasons and across phenological stages, with reduced values of 7.6 MJ ME - 9.9 MJ ME/kg DM when the plant is reproductive in summer due to the high levels of fibre and low digestible protein in the herbage PR (Burke et al., 2002; Fulkerson et al., 2007). Chicory has a ME concentration across seasons, ranging between 9.7 – 13.7 MJ ME/kg DM, with an average of approximately 11.5 MJ ME/kg DM, which is greater than average ME of PR (Hoskin et al., 1995; Barry, 1998a; Brown and Moot, 2004). The differences in ME between chicory and PR are more evident under dry summer conditions in summer. The high ME concentration of chicory during the dry summer conditions complements the traditional RGWC herbage that its poor nutritive composition often limits animal production.

2.2.5.1.5 Bioactive compounds

Chicory is rich in bioactive compounds. The predominant bioactive compounds in chicory are sesquiterpene lactones [**SL**; (Barry, 1998a)]. Sesquiterpene lactones are a group of natural occurring bioactive terpenoids which are part of the defensive mechanism of the plant against insect pest (Peña-Espinoza et al., 2018). The most common SL found in chicory are lactucin and lactucopicrin (Peña-Espinoza et al., 2018). The concentration of these SL previously observed in chicory was approximately 2% and 0.4% of DM in the leaves and roots respectively (Rees and Harborne, 1985; Peña-Espinoza et al., 2016). In ruminants, chicory feeding (50-70% of chicory dry matter in the diet) was shown to have antiparasitic activity against GI parasites in livestock (Clark et al., 1990b; Hoskin et al., 1999; Kidane et al., 2010). These studies linked the antiparasitic activity against GI parasites to the SL in chicory herbage. Sesquiterpene lactones have also been thought to be responsible for the bitter taste or aftertaste in some of the milk from cows grazing chicory (Barry, 1998a). However, 'Choice' forage chicory, bred from Grasslands Puna, has levels of approximately 70-85% lower than the levels found in Grassland Puna. Rumball et al. (2003a) stated that these low levels of SL are not expected to cause milk taint. No studies have compared the effects chicory cultivars on milk taint.

Chicory contains 1.7 to 4.2 g/kg of DM condensed tannins [**CT**; (Ramírez-Restrepo and Barry, 2005)]. The CT found in chicory are lower than the 14 g/kg of DM previously reported for plantain and higher than that of the traditional RGWC (Ramírez-Restrepo and Barry, 2005). The CT values in chicory are also lower than the 5 g/kg of DM required to protect proteins from rumen degradation (Jackson et al., 1996). However, Hoskin et al. (1995) and Kusmartono et al. (1996a) speculated that the presence of CT in chicory herbage was partially responsible for the improved performance of ruminants offered chicory compared with those offered RGWC. More studies are needed to explore the biological activity of condensed tannins found in chicory herbage.

Table 2.4. Crude protein and neutral detergent fibre concentrations (% of DM) of chicory compared with plantain and ryegrass across several studies.

		Crude protein			Neutral detergent fibre		
Season and Study	**Conditions**	**Chicory**	**Plantain**	**Ryegrass**	**Chicory**	**Plantain**	**Ryegrass**
Before vernalisation							
Spring							
Lebreveux et al., 2006		20.0	16.9		36.9	34.4	
Summer							
Lee et al., 2015a	250 cm	28.1	28.1		28.0	30.0	
	350 cm	26.9	26.3		28.0	40.0	
	450 cm	23.8	16.9		28.0	33.0	
Lebreveux et al., 2006		20.2	16.9		31.8	33.6	
Autumn							
	250 cm	28.1	25.0		23.0	25.0	
Lee et al., 2015a	350 cm	26.3	20.0		22.0	26.0	
	450 cm	21.9	16.9		23.0	26.0	
Minnee et al., 2018		19.4		23.8	20.1		42.3
After vernalisation							
Spring							
Box et al., 2017b	180 kg N/ha/year	16.9	15.0	15.0	17.1	23.5	41.4
	450 kg N/ha/year	18.8	17.5	19.4	21.3	16.0	40.2
Lee et al., 2015a	250 cm	23.1	16.9		25.0	28.0	
	350 cm	18.1	13.8		25.0	32.0	
	450 cm	16.3	13.1		23.0	35.0	
Lebreveux et al., 2006		16.3	14.4		39.6	40.6	
Summer							
Box et al., 2017b	180 kg N/ha/year	16.9	13.8	11.9	22.9	30.3	45
	450 kg N/ha/year	20.6	15.6	15.6	22.6	29.4	45.5
Lee et al., 2015a	250 cm	20.0	18.8		25.0	32.0	
	350 cm	18.8	16.9		29.0	32.0	
	450 cm	16.3	16.3		31.0	36.0	
Lebreveux et al., 2006		19.4	16.3		35.5	39.9	
Minnee et al., 2017		19.4	18.1	20.6	23.1	31.1	48.5
Li et al., 1997b	Leaf	25.0					
	Stem	9.4					
Brown et al., 2003	Leaf	17.5					
	Stem	7.5					
Autumn							
Box et al., 2017b	180 kg N/ha/year	20.6	18.8	17.5	18.6	23.5	44.3
	450 kg N/ha/year	21.9	20.0	21.3	22.1	23.8	43.5
Lee et al., 2015a	250 cm	25.0	21.3		23.0	29.0	
	350 cm	21.9	20.0		23.0	25.0	
	450 cm	21.9	18.1		23.0	26.0	
Martini et al., 2017	180 kg N/ha/year	19.4	17.5	15.6			
	450 kg N/ha/year	20.6	17.5	16.3			
Averages		**20.2**	**18.1**	**17.6**	**25.6**	**30.0**	**43.8**

2.2.5.1.6 Fatty acids

While diet PUFA are extensively biohydrogenated in the rumen, it has been shown that increasing their concentration in the diet of dairy cows can substantially improve both their concentration and that of CLA in ruminant-source foods (Jenkins et al., 2008; Elgersma, 2015; Toral et al., 2018). As a result, it is desirable to feed diets high PUFA to increase their concentration in the milk. At the farm level, it is possible to increase the concentration of functional FA in milk through supplementation with novel forage herbs with high PUFA content (Lourenço et al., 2008). Linoleic (**LA**; C18:2 c9, c12) and α-linolenic acid (ALA; C18:3 c9, c12, c15) in the herbage are the two main precursors of these beneficial FA in ruminant products (Lock and Bauman, 2004). In Switzerland, Puna chicory herbage sown with ryegrass (at 80% chicory and 20% ryegrass) contained 36% higher concentrations of LA than pure swards of ryegrass (Kälber et al., 2011). In southwest Victoria, Australia, an average of 5.38 mg/g of DM was obtained in pure swards of chicory during the spring, which was greater than the average of 2.10 mg/g of DM for PR (Muir et al., 2014). In the same experiment, ALA did not differ between chicory and PR herbages (Muir et al., 2014). However, in summer Muir et al. (2015) found greater ALA concentrations in chicory herbage than PR herbage (Table 2.5).

The composition of FA in the herbage is driven as much by forage type as by management practices (Dewhurst et al., 2006; Lourenço et al., 2008). Gazing management strategies affecting the FA concentration of forages could influence the PUFA content of milk. Information available in the literature suggest that the levels of LA and ALA in PR herbage varies considerably, depending on growth stage and management practices such as defoliation interval and severity. Growth stage and defoliation interval affect the morphology of the crop and therefore, the herbage FA composition (Dewhurst et al. 2001; Elgersma et al. 2003a). While these changes are well documented for PR, studies aiming at quantifying and describing such trends in chicory forage are lacking.

Other studies have demonstrated diurnal fluctuations of the total and individual FA in the herbage, which could potential affect the FA profile in the milk. Browse et al. (1981) noted an increase in the concentration of ALA and oleic acid in leaf blades of spinach and maize. Likewise, Gregorini et al. (2008) found a 22 and 13% increase in oleic acid in orchard grass and meadow fescue, respectively. These changes were associated with the increase in plant photosynthetic activity in the leaves as FA metabolism and biosynthesis are greater in actively growing leaves (Browse et al., 1981). There is no information regarding diurnal fluctuation of individual FA chicory herbage. The knowledge of these potential changes would help improve grazing management practices aimed at increasing functional FA in ruminant products.

Table 2.5. Individual fatty acid profile of chicory herbage compared with ryegrass across several studies.

	Reference	Clapham et al., 2005	Kälber et al., 2011	Kälber et al., 2014		Muir et al., 2014	Muir et al., 2015
	Conditions	Greenhouse	Vegetative	Vegetative	Reproductive	Reproductive	Reproductive
Fatty acid	**Units**	mg/g of DM	g/kg total FAME	g/kg DM	g/kg DM	mg/g of DM	mg/g of DM
Lauric (C12:0)	Chicory	0.0	58.3	0.0	0.0		
	Ryegrass	0.0	58.9				
Myristic (C14:0)	Chicory	0.4	34.6	0.1	0.1	0.12	0.2
	Ryegrass	0.6	42.1			0.13	0.1
Palmitic (C16:0)	Chicory	6.0	147.3	3.7	3.3	4.18	4.1
	Ryegrass	6.4	152.0			3.22	2.0
Palmitoleic (C16:1 c9)	Chicory	0.9	41.0	0.4	0.7	0.27	0.2
	Ryegrass	0.7	45.8			0.3	0.1
Stearic (C18:0)	Chicory	0.2	ND	0.3	0.3	0.28	0.5
	Ryegrass	0.3	15.3			0.35	0.3
Oleic (C18:1 c9)	Chicory	0.7	48.0	0.5	0.3	0.43	1.4
	Ryegrass	1.1	37.1			0.34	0.7
Linoleic (C18:2 c9,12)	Chicory	6.0	228.0	4.7	3.6	5.38	9.5
	Ryegrass	7.6	168.0			2.1	1.6
α-Linolenic (C18:3 c9,12,15)	Chicory	28.8	442.7	9.9	7.2	10.67	4.5
	Ryegrass	31.0	456.3			11.1	1.5

2.2.5.2 Inorganic compounds

2.2.5.2.1 Minerals

Tables 2.6-2.7 summarises macro- and micro minerals present in chicory and perennial ryegrass herbages across diets. Across studies, chicory contained similar or greater concentrations of macronutrients such as phosphorus, potassium, calcium, manganese, sodium and sulphur (Table 2.6). Micronutrients manganese, copper, zinc, boron and iron were significantly greater in chicory herbage than RGWC across several studies (Table 2.7).

Table 2.6. Macronutrient [phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na) and sulphur (S)] content (g/kg DM) of chicory and perennial ryegrass herbage across several studies.

Study	Forages	P	K	Ca	Mg	Na	S
Wilman and Rile, 1993	Chicory						
	Ryegrass	5.4	42.4	6.4	2.6	3.3	
Wilman and Derrick, 1994	Chicory						
	Ryegrass	4.1	41.2	5.4	1.8	3.5	
Crush and Evans, 1990	Chicory	3.3	68.7	13.2	2.8	3.5	
	Ryegrass						
Scales, 1995	Chicory	3.4	36.4	14.9		2.1	3.9
	Ryegrass	3.6	25.5	6.6		0.8	2.8
Barry et al., 2001	Chicory	2.6	15.8	11.7	3.2	5.4	2.8
	Ryegrass	3.1	11.7	5.6	2.2	1.8	2.2
Sanderson et al., 2003	Chicory	4.7	36.0	18.0	4.8		
	Ryegrass						
Harrington et al., 2006	Chicory	6.6	38.0	11.8	3.9	5.9	6.3
	Ryegrass	3.7	38.0	4.2	1.7	1.8	3.5
Jacobs and Ward, 2010	Chicory	3.5	37.6	15.0	4.5	13.0	4.2
	Ryegrass						
Marley et al., 2013	Chicory	3.4	16.5	13.4	4.4	3.4	1.9
	Ryegrass	4.0	28.0	6.0	2.0	3.0	3.0
Averages	**Chicory**	**3.9**	**35.6**	**14.0**	**3.9**	**5.6**	**3.8**
	Ryegrass	**4.0**	**31.1**	**5.7**	**2.1**	**2.4**	**2.9**

Table 2.7. Micronutrient [manganese (Mn), copper (Cu), zinc (Zn), boron (B), selenium (Se), cobalt (Co) and iron (Fe)] content (g/kg DM) of chicory and perennial ryegrass herbage across several studies.

Study	**Forages**	**Mn**	**Cu**	**Zn**	**B**	**Se**	**Co**	**Fe**
Scales, 1995	Chicory		11.0				0.5	
	Ryegrass		6.0				0.8	
Barry et al., 2001	Chicory	201.0	10.5	55.5		0.1	1.4	
	Ryegrass	183.0	7.6	45.2		0.1	0.6	
Morehead et al., 2002	Chicory							
	Ryegrass		11.3			4.8		
Sanderson et al., 2003	Chicory	170.0	32.0	45.0	33.0			
	Ryegrass							
Harrington et al., 2006	Chicory	161.0	18.6	57.7	38.3	0.0	0.3	167.0
	Ryegrass	99.0	7.9	22.0	19.0	0.0	0.2	151.0
Averages	**Chicory**	**177.3**	**18.0**	**52.7**	**35.7**	**0.1**	**0.7**	**167.0**
	Ryegrass	**141.0**	**8.2**	**33.6**	**19.0**	**1.6**	**0.5**	**151.0**

2.3 Animal performance

2.3.1 Grazing behaviour and dry matter intake

The grazing behaviour (grazing and rumination intensities) of dairy cows is related to forage type and time of day, which ultimately determines the feeding value of the herbage. Kusmartono et al. (1996a) measured similar time spent grazing, but 56% less time ruminating by deer offered chicory than deer offered RGWC. This result was supported by the findings of Gregorini et al. (2013), in which daily time spent grazing was similar, but daily time spent ruminating was 20% less for cows on chicory than those on RGWC. Gregorini et al. (2013) further showed that feeding chicory at 60% of total DMI reduced ruminative chewing by up to 20% compared with feeding the control RGWC herbage. Barry (1998) speculated that chicory could be broken down very rapidly in the rumen because of its higher ratio of NFC to structural carbohydrates and low concentration of silicon. A recent study reported that chicory herbage required 20% of the energy to macerate than RGWC, which eased the manipulation of chicory herbage into a bolus, thus reducing the requirement for chewing (Minnee et al., 2019). Indeed the rumen fractional degradation rate and fractional outflow rate was nearly two times greater in deer fed chicory than deer fed RGWC

(Kusmartono et al., 1996b, 1997). Burke et al. (2000) suggested that the rapid rumen degradation of chicory would allow for greater DMI than RGWC.

However, the effect of chicory compared with the traditional RGWC on DMI has not been unanimous. In two grazing experiments in Australia in spring and summer, no differences in DMI were found between cows offered pure swards of reproductive chicory and those offered PR (Muir et al., 2014, 2015). Similarly, in an indoor experiment carried out in Hamilton, New Zealand, where late lactating cows were offered PR diets or diets containing 20-40% vegetative chicory, Minneé et al. (2017) found similar DMIs across diets. In the above studies, the authors attributed the lack of chicory effect on DMI to the low DM content of the herbage. Tinworth et al. (1999) conducted an indoor study to evaluate the impacts of wilting chicory to reduce its bulkiness on DMI of castrated male red deer. Deer fed wilted chicory had ~16% higher DMI intake than those fed fresh chicory.

On the other hand, studies at Massey University, reported 56%, 26% and 15% greater voluntary feed intake from deer grazing chicory than those grazing RGWC during summer, autumn, and spring, respectively (Kusmartono et al., 1996b; a, 1997). These findings are in agreement with the research of Chapman et al. (2008), in which dairy cows on vegetative chicory ingested almost twice as much DM than those on grass based diets in Australia in summer. Minneé et al. (2017) speculated that the animals that had higher DMI than grass based diets (Kusmartono, 1996a; Chapman et al., 2008) must have ingested a larger number of chicory boli to compensate for the low DM content of the feed and attain their daily DMI requirements. Indeed, Gregorini et al. (2013) found a greater number of ingestive mastications by cows on chicory than cows on RGWC even though they had similar daily grazing time, suggesting the rate of swallowing individual boli of chicory was more rapid than for ryegrass. Nevertheless, the discrepancies in literature requires compiling numerous empirical studies that describe grazing behaviour and DMI of dairy cows offered chicory compared with the traditional RGWC herbage in situ.

2.3.2 Milk production

The inclusion of chicory into the feeding systems have been found to yield conflicting results on milk production (Table 2.8). Muir et al. (2015, 2014) found similar milk production response from cows grazing PR-based and chicory-based herbage (at 80% of the diet) in Australia in spring and summer (Table 2.8). The similarities in milk production from cows grazing chicory to those grazing PR was accredited to the higher stem material in the herbage, which increased the fibre content of chicory; and therefore, affected DMI and milk production responses. All treatment cows were also supplement with alfalfa hay, which might have diluted the beneficial effects of chicory (Muir et al., 2014).

Another study conducted in summer in Australia (Chapman et al., 2008) observed significantly greater milk production from cows offered chicory based diets compared with those offered a grass based diet (18.8 vs 9.8 l/cow/day. A subsequent study conducted in Hamilton, New Zealand in summer, noted a greater milk production response from cows offered chicory diets when ME was greater for chicory than PR herbage (Minneé et al., 2012). When ME was not different between chicory and PR herbages, milk production was also not different between treatments. This suggest that offering chicory to dairy cows would be beneficial during periods when the quality of the traditional PR is low. This premise was later collaborated by the findings of Minneé et al. (2017), who observed that the milk production response of feeding chicory compared with PR varied between measurement weeks due to variation in DMI and nutritive value of the respective forages (Table 2.8). In Week I, when DMI was 10% less for dairy cows offered chicory than those offered PR, milk production was not different between diets. In Week II, when DMI was not different, cows offered chicory produced 19% more milk than those offered PR, reinforcing the benefits of offering forages with a high feeding value (Waghorn et al., 2007). Nonetheless, given the inconsistent milk production results in the literature, long term studies are required to investigate the direct effects of including pure swards of chicory in dairy-farming systems on milk production. These studies are needed in late spring/summer when the herbage quality of the taditional RGWC is low.

2.3.3 Milk Composition

2.3.3.1 Milk Protein

The effect of chicory feeding on the main milk components is minor (Table 2.8). There were no differences in milk protein percent from cows grazing PR herbage and those grazing chicory herbage in three studies conducted in Australia and New Zealand (Muir et al., 2014, 2015; Minneé et al., 2017). However, differences in milk volume led to milk protein yield of cows grazing chicory-based herbage being 1.8 times greater than for cows grazing grass-based herbage (Chapman et al., 2008). Totty et al. (2013) also found greater milk protein yield from cows on diverse pasture mixture containing chicory than cows on RGWC at similar milk volume. An increase in daily milk protein yield at similar milk yield is desirable, as it indicates greater partitioning of N to milk in the form of protein (Totty et al., 2013). Partitioning more dietary N to milk or muscle instead of other pools such as urine may reduce the environmental impacts associated with livestock farming (Tamminga, 1992). An increase in milk protein may also provide an economic benefit to farmers who are paid on a milk solids basis.

Table 2.8. Milk yield (kg/cow), milk solids (fat + protein; kg/day), milk fa (%), milk protein (%) and milk lactose (%) of dairy cows offered chicory-based diets in comparison with perennial ryegrass (RGWC)-based diets across several studies.

Study	Season and country	Forages	Milk yield	Milk solids	Fat	Protein	Lactose
Wagh, 1998	Summer	Chicory	10.75	0.93	5.03	3.49	
	New Zealand	RGWC	10.38	0.87	4.97	3.53	
Chapman et al., 2008	Summer	Chicory	18.80	1.41	4.12	3.45	
	Australia	RGWC	9.80	0.77	4.43	3.59	
Minnee et al., 2012[1]	Autumn	Chicory	12.60	1.02			
	New Zealand	RGWC	9.90	0.96			
Minnee et al., 2012[2]	Autumn	Chicory	14.90	1.03			
	New Zealand	RGWC	15.70	1.01			
Kalber et al., 2011	Switzerland	Chicory	19.70		4.61	3.18	4.65
	Summer	RGWC	20.50		4.55	3.16	4.65
Kalber et al., 2014	Switzerland	Veg[3] Chicory	19.30		4.50	3.47	4.72
	Summer	Rep[3] Chicory	14.90		4.78	3.59	4.72
Muir et al., 2014	Spring	Chicory	28.00	1.92	3.65	3.30	
	Australia	RGWC	28.50	1.90	3.66	3.28	
Muir et al., 2015	Summer	Chicory	12.00		4.73	3.62	
	Australia	RGWC	12.00		4.30	3.54	
Minnee et al., 2017	Summer	Chicory	12.60	1.03	4.90	3.46	4.58
Week 1	New Zealand	RGWC	12.20	1.01	4.93	3.42	4.53
Minnee et al., 2017	Summer	Chicory	12.60	1.02	4.89	3.51	4.55
Week 2	New Zealand	RGWC	9.90	0.83	5.06	3.42	4.45

[1]Poor pasture estimated ME (9.6 MJ/kg DM); [2]Moderate pasture estimated ME (10.5 MJ/kg DM); [3]Veg: chicory at vegetative stage, Rep: chicory at reproductive stage.

2.3.3.2 Milk Fat

The low NDF concentration of chicory herbage has been suggested to result in milk fat depression (Waugh et al., 1998). However, Muir et al. (2014; 2017) found that dairy cows grazing chicory or PR exhibited similar milk fat percentage (Table 2.8). In the reported study, cows on chicory diets selected diets with an NDF concentration closer to 25% (Muir et al., 2014). Muir et al. (2015) concluded that despite chicory having lower NDF% than the recommended 30-40% for lactating dairy cows diets (NRC, 2001), feeding chicory diets at 80% of the diet does not cause milk fat depression. Minneé et al. (2017) also found no difference between milk samples in terms of milk fat percentage from dairy cows fed PR or chicory-based diets at 20 or 40% of the diet (Table 8).

Over the last decade, a few studies have explored the effect of chicory feeding on individual milk FA composition due to the surge in consumers concerns over the negative effects of some individual milk FA on human health. Saturated FA have been linked with health complications such as obesity and cardiovascular diseases. While polyunsaturated FA have been reported to have many beneficial human-health related effects. For example, consumption of ALA was reported to exert neuroprotective, anti-inflammatory, and antidepressant properties in humans (Blondeau et al., 2015). In the body, ALA is also converted to eicosapentaenoic acid, a FA that is known for its cardio-protective and other human health benefits (Rajaram, 2014). Conjugated linoleic acid is another important FA that has been linked with positive health benefits in humans, reducing diabetes, cancers and obesity amongst others (Jenkins et al., 2008; Toral et al., 2018). Therefore, diets that increase the concentration of CLA and ALA in ruminant-source foods are sought.

Petersen et al. (2011) found 38% lower concentrations of CLA in milk of cows fed chicory-grass based diets than those fed clover-grass based diets. Likewise, Muir et al. (2014) reported that chicory-fed cows had 40% lower levels of vaccenic acid (**VA;** C18:1 t11**)**, correlating with 30% lower CLA levels in milk of dairy cows as compared with those cows on PR. However, the authors attributed this to the inclusion of alfalfa hay in chicory diets (Muir et al. 2014). Hay has been described to affect the concentration of certain FA in milk, including VA and CLA (Chilliard et al., 2001). Subsequently, Muir et al. (2015) conducted a grazing experiment to compare the effect of chicory-based diets and PR-based diets of milk FA. In their experiment, all treatment cows were supplemented with equal proportions of alfalfa hay. Muir et al. (2015) reported significantly higher concentration of VA and CLA in milk of cows grazing chicory diets than those grazing grass-based diets when all cows were supplemented with alfalfa hay. Other studies reported no differences in VA and CLA in milk of cows (Kälber et al., 2011) and ewes (Rodríguez et al., 2020) on chicory diets as compared with those on grass diets. The inconsistence in literature warrants further investigations.

Kälber et al. (2011) noted an 18.3% and 21.6% increase in milk concentration of LA and ALA from chicory-fed dairy cows than PR-fed dairy cows at Day 11 to 20 after the start of treatment in Switzerland. In a summer grazing experiment in Denmark, Danish Holstein Friesian dairy cows were fed a herb-based diet or a clover based diet for 14 days (Petersen et al., 2011). The herb diet contained (on DM basis) 43% chicory, 21% plantain and 11% salad burnet amongst other herbs. The clover-grass diet contained 78% white clover, 21% perennial ryegrass and 1% weeds. The milk from dairy cows fed the herb based-diets contained 86% and 75% greater concentrations of LA and ALA, respectively, than the milk from cows fed the white clover-based diets. Recent studies in dairy cows in Australia (Muir et al., 2014, 2015) were in agreement with these findings; reporting greater concentrations of LA (+22-74%) and ALA (+71-97%) in milk of dairy cows offered chicory based diets as compared with cows on grass based diets.

While there is clear evidence that chicory feeding enhances the concentration of functional FA, LA and ALA, in ruminant source-foods, the mechanisms leading to the increased PUFA from chicory diets are still largely unknown. Understanding the mechanisms would help improve grazing management strategies that would enhance the concentration of the desirable FA in the ruminant source-products. In order to understand how the proportion of the desired FA in milk may be enhanced, the metabolism of FA should be understood.

There are four major sources of milk FA, with the first being *de novo* synthesis in the mammary gland. *De novo* synthesized FA accounts for nearly 50% of milk fat and tend to be short-medium chain acids (C4:0 to C14:0) plus half of C16:0 FA. These FA are mainly determined by animal genetics (Knutsen et al., 2018). Acetate and β- *hydroxybutyrate* are the main precursors for the synthesis of these FA in the mammary gland (Shingfield et al., 2013). Fatty acids can also be sourced from lipolysis and the mobilization of body fat. These may account for 5% in a well-fed animal to over 20% of milk FA in early lactation when cows are in a negative energy balance (Bauman and Griinari, 2003; Lock and Bauman, 2004). Generally, cows in a negative energy balance during early lactation reduce the concentration of C5–15 but elevate the concentration of C16:0 and C18:0, reflecting energy shortage and reallocation of C3 components in the FA *de novo* synthesis, as well as body fat reserve mobilization (Stoop et al., 2009). The odd and branched chain FA represents FA of microbial origin (Vlaeminck et al., 2006). Their concentration in the milk varies markedly. The fourth, and most studied source of milk FA is the animal diet. The FA in milk perceived to have healthy benefits are long chain FA of diet origin, and therefore feeding strategies that will enhance their rumen by-pass have been studied extensively.

ALA and LA are the two major FAs that their metabolism has been studied extensively. Figure 2.3 illustrates the generalised scheme of biohydrogenation of LA and ALA in the rumen. The metabolism of lipids in the rumen involves two consecutive stages: lipolysis and biohydrogenation. Lipolysis is a prerequisite of biohydrogenation. Upon entering the rumen, lipase, produced mainly by ruminal bacterium *Anaerovibrio lipolytica*, hydrolyses the ester linkages in the complex lipids causing the release of non-esterified FA with free carboxyl groups in the ruminal digesta that can be hydrogenated by other rumen microbes (Dawson et al., 1977). The rate and level of hydrolysis of the ingested FA is generally high [>85%; (Lock and Bauman, 2004)], depending on factors such as ruminal pH, feed intake level and rumen retention time of lipids (Lock and Bauman, 2004; Elgersma, 2015; Toral et al., 2018).

After lipolysis, the free unsaturated FA are rapidly hydrogenated by the rumen microbes to yield saturated FA, primarily stearic acid (C18:0) as the end-product of rumen biohydrogenation (Figure 2.3). Rumen biohydrogenation is required to reduce the toxicity of PUFA to the ruminal microbes (Lock and Bauman, 2004). The extent and rate of biohydrogenation in the rumen is extensive, with 70–95% and 85–100% of LA and ALA hydrogenated, respectively (Lock and Bauman, 2004). Under certain conditions, higher level of LA and ALA escape rumen lipolysis and biohydrogenation, increasing their concentration in the milk. Dry matter intake, FA composition of ingested feeds and the extent of rumen biohydrogenation influence the amount of FA recovered in the milk (Elgersma, 2015).

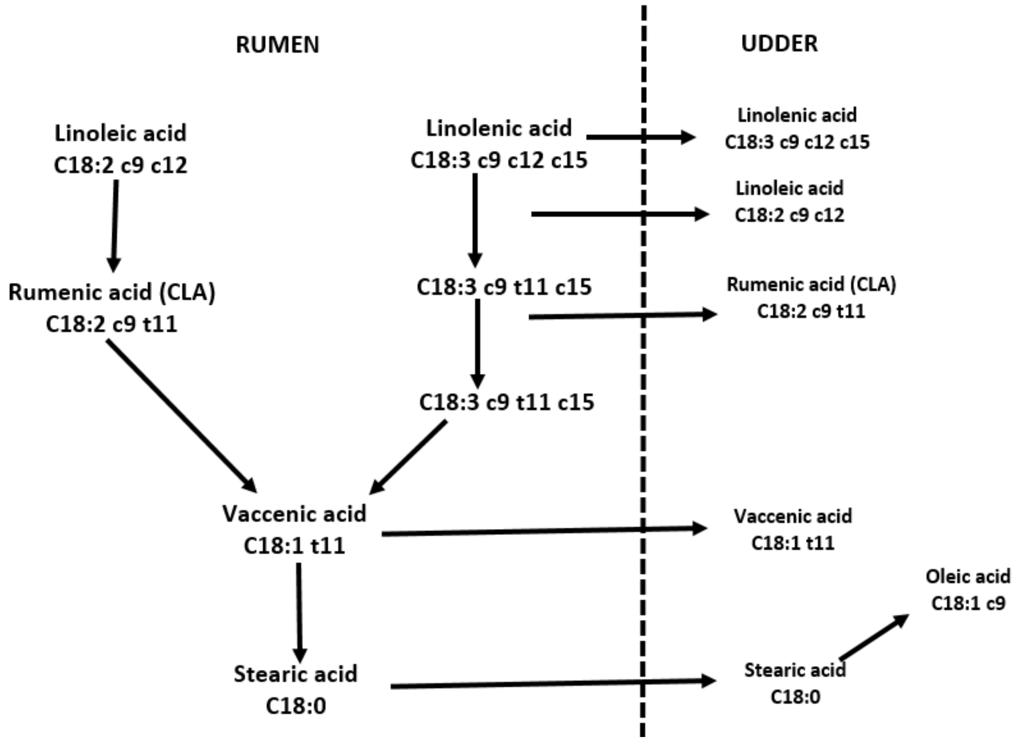

Figure 2.3. Generalised biohydrogenation pathways of linoleic (C18:2 c9 c12) and α-linolenic acid (C18:3 c9 c12 c15) in the rumen and udder. Adapted from Lock and Bauman (2004).

2.3.3.2.1 Manipulation of milk fatty acid composition in pastoral systems

It is well documented that milk from cows grazing fresh herbage is greater in PUFA concentration than milk from cows kept indoors and fed diets rich in concentrates or conserved forages such as maize silage or hay. This has been linked to the greater concentration of PUFA in the diets of fresh herbages than conserved forages or concentrates (Lourenço et al., 2008; Palmquist, 2009). Likewise, the increase in LA in cows fed chicory compared with RGWC in previous studies was linked to the greater concentration of LA in the chicory herbage (Kälber et al., 2011; Petersen et al., 2011; Muir et al., 2014, 2015). However, while all diets based on chicory increased ALA in milk fat as compared with ryegrass diet, the corresponding swards were not generally richer in ALA (Table 2.7). This suggest that other mechanisms that improved the apparent transfer of PUFA from chicory herbage to milk are probably involved. Several hypotheses can be made to explain this increase in transfer efficiency in PUFA from chicory herbage to milk.

The first hypothesis is faster rumen passage rate due to the lower fibre content, which may reduce microbial contact with dietary FA thus limiting biohydrogenation of herbage PUFA (Lourenço et al., 2008; Elgersma, 2015). This premise is supported by Dewhurst (2003), in which rumen passage rate was higher in dairy cows fed clover-based diets than those fed grass-based diets, which increased the proportion of α-linolenic acid passing through the rumen. Although it has been shown that chicory increases rumen passage rate (Kusmartono et al., 1997) these studies have been performed on deer kept indoors, and therefore studies on dairy cows grazing chicory *in situ* are desired.

Another hypothesis is reduced rumen biohydrogenation due to the presence of secondary plant metabolites in chicory herbage. The SL found in chicory herbage have been found to have antimicrobial properties, which may affect the activity of the microorganism responsible for biohydrogenation, leading to the accumulation of PUFA in the rumen. Chicory contains more CT than RGWC herbage. It has been established that CT found in herbages may form large protective complexes with fibre and proteins which may alter rumen functioning, disrupting ruminal biohydrogenation (Lourenço et al., 2008). This may increase the amount of PUFA escaping rumen biohydrogenation, leading to increased recovery of herbage PUFA in milk.

A third hypothesis is reduced lipolysis at lower rumen pH. In a review, Chilliard et al. (2001) reported a 35% to 50% decrease in ruminal biohydrogenation of PUFA, such as LA and ALA when concentrates formed more than 70% of the diet, a result of reduced lipolysis at lower rumen pH. Diets high in readily fermentable carbohydrates are known to increase total VFA and reduce pH during ruminal fermentation, limiting lipolysis, and thus, biohydrogenation (Chouinard et al., 1999; Kolver and De Veth, 2002). Chicory contains more readily fermentable carbohydrates than RGWC (Minneé et al., 2017), which may increase the concentration of total VFA and lower rumen pH during ruminal fermentation, thus modifying the milk FA profile. By contrast, no studies have linked chicory feeding with rumen functioning, and therefore milk FA composition. Comparing the rumen function of cows grazing chicory with those grazing RGWC *in situ* could provide insights into rumen FA metabolism that could help design grazing management practices that could manipulate the milk FA composition of ruminant-sourced products.

2.3.3.3 Milk urea nitrogen

The effect of grazing different herbage types on milk urea nitrogen (**MUN**) has been investigated. Milk urea nitrogen from cows offered diverse swards containing forage herbs plantain and chicory was reported to be lower compared with that from cows offered the traditional RGWC (Bryant et al. 2017; Totty et al. 2013). The diverse swards that reduced MUN also reduced the concentration of urinary N, indicating an environmental benefit from feeding the diverse swards.

Milk urea nitrogen is generally used as a biomarker of the efficiency for milk production (milk N/N intake) in lactating cows (Huhtanen et al., 2015). Substantial variation in MUN across different forages highlights the potential for improving efficiency of N utilisation and therefore the proportion of N excreted in urine. Overfeeding of herbage CP has been highlighted as the major reason for high MUN. Therefore, careful consideration of herbage CP levels of the novel forages and overall CP intake from the animals grazing these forages is vital for optimizing N use efficiency (**NUE**) in order to minimize N loss. Although cows fed diverse swards containing chicory have consistently reduced MUN compared with those fed the traditional RGWC, information on MUN from cows offered pure swards of chicory is scarce. Chicory herbage generally contains higher CP concentration than the traditional RGWC (Table 2.4), and therefore research around protein digestion, metabolism and urea recycling and subsequent effect on MUN needs to be addressed to predict its potential effect on the environment.

2.3.4 Urine N excretion

There is a considerable increase in societal scrutiny on pastoral livestock farming due to the perceived environmental impact in terms of excessive N discharges to waterways. Consequently, regional councils throughout New Zealand have placed limits on the amount of nitrate leached from agricultural land (Ministry for the Environment 2014). The Government has set a national target of 90% of specified rivers and lakes to be safe for primary contact by 2040 (Ministry for the Environment 2017). Animal excreta, especially urine, is one of the major sources of N contaminating the nearby agricultural waterways (Wachendorf et al., 2005). This is mainly because the N deposited in urine patches [200 to 2000 kg/ha N (Selbie et al., 2015)] is often at a rate too high for plants to use, resulting in N loss through leaching when drainage occurs (Scholefield et al., 1993; Wachendorf et al., 2005). In temperate regions, this is often exacerbated during the cool season period when growth of the traditional RGWC is low, and therefore N uptake. Several strategies are being explored to mitigate N losses to achieve regulatory limits in pastoral systems (Bryant et al., 2019). The proposed strategies span from including forage species that grow more during cool season (Malcolm et al., 2018; Woods et al., 2018), or forages with a deep rooting system to capture N in soil (Malcolm et al., 2014), to forages that alter N partitioning in animals, thus reducing N concentration in the urine (Totty et al., 2013; Box et al., 2017a). Reducing UN concentration has emerged as a promising strategy to reduce N loss in pastoral systems (Di and Cameron, 2002). This is because the risks of nitrate leaching at the level of urine patch are dependent on N load (Di and Cameron, 2002), which is reliant on urine volume and urinary N concentration (Li et al., 2012). As an example, Ledgard et al. (2015) reported a 65% reduction in leaching rates when the concentration of N in the urine was reduced by 50%.

Several studies have explored the in vivo effects of alternative forage species on UN concentration of ruminants. From studies including forage herbs in the diet, significant reductions of N concentration in the urine have been reported (Totty et al., 2013; Bryant et al., 2017). However, many of the grazing studies contain both chicory and plantain, so it was not clear whether the benefits were because of chicory or plantain. Plantain studies that excluded chicory resulted in significant reductions of N concentration in the urine of sheep or dairy cows (Box et al., 2017; O'Connell et al., 2016; Woodward et al., 2013). For example, in two short term experiment in the Waikato region of New Zealand, including narrow leafed plantain in a perennial ryegrass or tall fescue diet at 46%–70% of DMI significantly lowered UN concentration by 38% in summer and 21% in spring (Dodd et al., 2019). These findings strongly suggest that plantain feeding directly reduces UN concentration. Recently the environment regulatory tool, OVERSEER, has been revised to include plantain and a series of assumptions relating to changes in urinary N load, and N partitioning to dung to predict farm level N loss (Shepherd 2020). Plantain is a high moisture, low fibre forage, with plant secondary compounds which may influence protein degradation and urea metabolism. However, chicory also possesses similar characteristics to plantain and Minneé et al. (2017) found that including up to 40% of chicory into the RGWC diet of dairy cows reduced UN concentration by 38% compared with the control RGWC diet. This also suggest the potential of chicory to reduce the environmental impacts associated with pastoral livestock farming systems. Although this study has shown promising results at reducing UN concentration indoors, there is a lack of information outdoors and at different seasons. The mechanisms by which alternative forages reduce the concentration of N in the urine have been reviewed extensively (Edwards et al., 2007; Dijkstra et al., 2013; Bryant et al., 2019). Briefly, the decline in UN concentration is associated with (1) reduced N intake (2) balanced N and energy supply and (3) urine dilution.

2.3.4.1 N intake

High N intake is known as the principal driver of N loss in cattle, mainly due to its direct effect on N use efficiency [NUE (Yan et al., 2007; Huhtanen et al., 2008)]. An increase in N intake decreases NUE as the majority of the additionally N is not utilised to support milk production or muscle growth, but lost in the animal excreta (Dijkstra et al., 2013). In a meta-analysis, Kebreab et al. (2010) found that lowering N intake from 600 to 300 g/day increased NUE from 0.25 to 0.30, respectively, and proportionally decreased N lost in faeces and urine. Nitrogen intake is reliant on DMI and diet N concentration. Generally, CP concentration between 14 to 18% CP of DM, depending on stage of lactation, is required in the diet of dairy cows to support milk production (NRC, 2001). The concentration of CP of the predominant sward species used in intensively managed dairy systems in temperate regions is often too high (Table 4), which occurs most typically in autumn. This high CP in the herbage coincides with the end of lactation, where the CP requirements of the cows are low. This surplus CP in the diet increases levels of urea in the urine and blood, as well as the percentage of urine urea-N to total urinary N (Tamminga, 1992). Ultimately forages with moderate CP are sought to reduce the amount of N excreted in urine.

In an early-summer grazing experiment at Lincoln University, mid-lactation dairy cows were offered either the control RGWC sward or a diverse sward containing chicory, plantain, lucerne and RGWC forages for nine days (Edwards et al., 2015). Cows offered the diverse diet selected herbage with lower N concentration than cows offered the control diet (3.1% vs 3.6% N of DM), and the concentration N in the urine was lower from cows grazing the diverse herbage than those grazing the control (4.9 vs 6.1 g N/L), a result of reduced UN concentration at lower N intake of the diet. However, there is also evidence that other forages having similar or high N concentration compared with RGWC can still reduce the concentration on N in the urine. In an indoor study at DairyNZ Farm at Hamilton, dairy cows were fed PR herbage as control or chicory herbage at 20 and 40% of the diet for 22 days (Minneé et al., 2017). Despite similar N content and N intake of chicory and RGWC-based diets, UN concentration was 38% lower in cows fed a diet consisting 40% chicory than those fed a RGWC diet. This suggest that there is not only one mechanism involved in reducing UN concentration.

2.3.4.2 Balancing N and energy supply

While N intake is a key factor in determining N excretion rates, the use of alternative forages to match dietary N and energy available has the potential to reduce total urinary N excretion and the concentration of N in the urine (Edwards et al., 2007). A higher ratio of readily available carbohydrates to dietary N is known to increases the quantity of N retained and reduce the quantity of N excreted via urine (Dijkstra et al., 2013). This is mainly related to the improvement in rumen supply and synchrony of energy with N which reduces rumen N losses and increases microbial protein synthesis (Kebreab et al., 2001). In a review, the use of high sugar PR was reported to improve NUE, which led to a decline in total N excreted in the urine (Edwards et al., 2007). However, there is also evidence that the use of diverse swards containing chicory, plantain, lotus, high-sugar PR, and white clover other than just the traditional RGWC can still improve the synchrony of energy with N, ultimately improving NUE and reducing total N excreted in the urine (Totty et al., 2013). Chicory forage has a higher ratio of readily fermentable carbohydrates to CP ratio than RGWC (Kusmartono et al., 1996a; Burke et al., 2006), which might improve energy and protein supply to meet microbial requirements, improve NUE and reduce the amount of N excreted.

2.3.4.3 Urine dilution

There is a substantial volume of information available of mitigation strategies based on dietary inputs and manipulations to lower UN concentration by diluting N intake (Dijkstra et al., 2013; Bryant et al., 2019). Lower concentrations of UN have been reported in experiments where urine volume increased (Dijkstra et al., 2013). To demonstrate the effects of urine volume on UN concentration, researchers fed salt to dairy cows to increase water intake (Spek et al., 2012; Ledgard et al., 2015). Cows supplemented with salt increased total water intake, which led to increased urination frequency and urine volume, resulting in a lower UN concentration at similar N intakes (Spek et al., 2012; Ledgard et al., 2015).

The intakes of minerals, such as sodium (Na) and potassium (K), have major impacts on daily urine volume (Nennich et al., 2006). In an indoor experiment, in which dairy cows were offered isonitrogenous and isoenergetic diets, De Campeneere et al. (2006) compared the effects of feeding grass silage-based diets rich in Na and K to that of maize silage-based diets with much lower Na and K concentrations. Despite the similarities in DM content of the diets, cows offered diets rich in Na and K produced 2.4 times greater urine volume than those offered diets lower in these minerals (De Campeneere et al., 2006). However, total water intake was not reported in that study.

Alternatively, recent studies have demonstrated the potential use of forages with traits that have been identified to increase feed and total water intakes and therefore reduce the concentration of N in the urine (O'Connell et al., 2016; Box et al., 2017a). Plantain is one of the major forages that its effect on urine volume and UN concentration has been studied extensively. Just recently, in two grazing experiments that involved detailed monitoring of urination events and measurement of total urine volume using urine sensors in autumn and spring, in addition to urine spot sampling, it has been shown that cows offered plantain-based diets increased urination frequency and urine volume by an average of 42%, leading to lower UN concentration by an average of 54% compared with cows on RGWC (Box et al., 2017a). This is in agreement with the results from an indoor study which also showed increased urine volume from sheep offered plantain diets than those offered RGWC (O'Connell et al., 2016). The authors attributed the increase in urine volume to water diuresis caused by the high moisture content of plantain.

Although it has been shown that intake of high moisture forages affects water metabolism and urination patterns, these studies have been performed with only plantain, and therefore do not include chicory, a forage crop that is also high in moisture content and has the potential to reduce the concentration of N in the urine. In general, there is very little of the urination patterns of cows offered other forages on other forages other than plantain. This lack of information relates to difficulties of measuring water intakes, urination patterns, urine N excretion patterns of individual cows under grazing conditions. Studies on other forages that could alter urination patterns, diminishing N leached to ground water would help to plan feeding strategies and/or manipulate the botanical composition of managed swards, which subsequently will lead to reduced environmental impacts associated with the traditional pastoral system based on RGWC.

2.4 Conclusions and opportunities

a. Chicory is a highly productive forage crop, that is greater in nutritive value when compared with the traditional RGWC, particularly in summer. However, the development of reproductive stems in vernalised chicory plants together with the its limited persistence have been identified to have slowed its adoption into the pastoral system. Therefore, there is need to generate specific and efficient grazing management strategies that could control the development of stems in winter vernalised chicory plants while enhancing the persistence of the herb.

b. Chicory feeding to dairy cows offers potential benefits in improving milk production in summer while enhancing the concentration of functional FA in ruminant source products, more especially milk. However, the mechanism leading to the increased concentration of the functional FA in milk of chicory fed cows is not understood. Therefore, there is need for more information to understand the mechanisms leading to increased PUFA from chicory diets and the associated feeding management in order to capture the value of chicory as a means for improving product quality in terms of milk FA.

c. There is a lack of knowledge on the impact of feeding chicory and the associated grazing management practices on urination patterns, urinary N excretion and therefore environmental sustainability of including the herb into the traditional pastoral system.

Chapter 3

3. Forage herbs as an alternative to ryegrass-white clover to alter urination patterns in grazing dairy systems

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3.1 Introduction

Grazing livestock excrete most of their nitrogen (N) in urine and faeces (Ruzjerez et al., 1995) with over 60% of N excreted as urinary nitrogen [UN; (Kebreab et al., 2001)]. Research investigating the fate of soil mineral N under urine patches has shown that a large proportion of N is converted to nitrate and subsequently leached following drainage events (Di and Cameron, 2002). Accordingly, urine patches are regarded as the major contributors of N loss from grazed systems (Selbie et al., 2015; Luo et al., 2018). While reduction of total urinary N excretion has been targeted as a pathway to reduce farm level N losses, it has also been recognised that leaching risk can be managed by altering the N load [kg N applied to a urination patch; (Li et al., 2012)]. Urine N load is the product of UN concentration (g/L N of urine) and urine volume [L of urine per event; (Di and Cameron, 2002; Ledgard et al., 2009) and changes in N load signify variation in both diurnal patterns of feeding and the forage characteristics. Nitrogen loading to soil from cattle urination ranges from 200 to 2000 kg/ha N, averaging ~700 kg/ha N per urination event in traditional pastoral farming systems (Selbie et al., 2015). These rates exceed the threshold for plant uptake and so the surplus N is at risk of being lost. Consequently, mitigation strategies are being explored which alter individual animal urine volume patterns and UN excretion in order to modulate N losses in grazed systems.

Dilution of urinary N has been proposed as a means of reducing N loading to soils and, in some studies, mineral supplements such as sodium chloride (NaCl) have been fed to cows to increase water intake to dilute the urine (Ledgard et al., 2009; Spek et al., 2012). Alternatively, forages with low dry matter (DM), but high mineral content such as chicory (*Cichorus intybus* L.) or plantain (*Plantago lanceolata* L.) present an opportunity to manipulate the dietary features of grazing ruminants, to modulate urination behaviour and UN excreted into the environment. Pure swards of chicory and plantain have shown agronomic potential as alternative forages to traditional perennial ryegrass (*Lolium perenne* L.)/white clover (*Trifolium repens* L.) (RGWC) pastures for summer/early autumn pastures in intensive dairying systems located in temperate regions (Li and Kemp, 2005; Lee et al., 2015a). Both herbs, when sown in mixtures with grass and clover or sown as a monoculture (Neal et al., 2009; Nobilly et al., 2013), can accumulate similar or greater herbage yield than ryegrass/white clover swards, particularly during warmer seasons due to their deep root systems (McNally et al., 2015) and drought tolerance, and are expected to adapt easily into existing farm systems (Beukes et al., 2012; Pembleton et al., 2015). Consequently, there is growing interest in integrating herbs such as chicory and plantain into pastoral systems by either using them as a summer feed and/or transitioning them into permanent pastures. Moreover, several animal studies investigating chicory and plantain either as a mixture (Totty et al., 2013; Bryant et al., 2017) or, in the case of plantain, as the sole diet (Box et al., 2017a), have consistently shown reduced UN concentration from spot urine samples. These findings led to the hypotheses that those herbs had diuretic effects, explaining the lower UN when apparent N intakes were similar to the controls (O'Connell et al., 2016; Box et al., 2017a; Cheng et al., 2017).

However, the only study to report daily urine volumes of cows fed mixed swards containing chicory and plantain compared with control RGWC swards, did not detect differences in urine volume between treatments (Bryant et al., 2018). Bryant et al. (2018) did however, report changes in diurnal patterns of urination and suggested that the lack of larger differences in urination patterns was due to herbs constituting only a third of the diet. Thus the objective of the current research was to test the hypothesis that feeding herbs chicory and plantain, which are high in water and mineral content, as sole diets will cause a diuretic effect and alter urination behaviour without altering their productivity compared with traditional RGWC pastures.

3.2 Materials and methods

3.2.1 Experimental site

The experiment was conducted between 28 February and 15 March 2018 at the Lincoln University Research Dairy Farm (43°38′S, 172°28′E; 17m above sea level), with the approval of the Lincoln University Animal Ethics Committee (AEC #2018-06). The soil is classified as free-draining Templeton fine sandy loam soil (Hewitt 2010) with soil pH of 6.2 (1: 2.1 v/v soil–water slurry), Olsen phosphorus 29.7 mg/L, potassium 0.9, calcium 8.2, magnesium 1.1 and sodium 0.2 me/100 g as determined on 29 September 2017 to 150mm depth. No fertilizer was applied at establishment.

The experiment was a completely randomized design with three forage treatments and three replicates. The forages were: (1) chicory; (2) plantain, and (3) perennial ryegrass/white clover (RGWC) swards. The ryegrass (cv. Arrow AR1; 20 kg/ha) and white clover (cv. Weka; 3 kg/ha) swards were established in October 2013. Chicory (cv. Choice, 5.3 kg/ha) and plantain (cv. Tonic, 10 kg/ ha) swards were established in November 2017 following cultivation. All forages were grown under irrigation and, following establishment, chicory was grazed twice (mid-January and early-February 2018) and plantain grazed once (late-January 2018) by experimental animals to familiarise them with the forage. Preparation of pastures prior to the experiment was based on relative herbage growth rates and requirement for uniform feed quality. Approximately five weeks prior to experiment plantain areas were post graze mown to 3.5 cm and three weeks prior to the experiment, chicory and RGWC areas were pre-graze mown to 3.5 cm (Plate 3.1). Urea was applied at 50 kg N/ha to all areas three weeks prior to the experiment.

Plate 3.1. Chicory herbage pre-mowed in preparation for the grazing experiment at LURDF, Canterbury NZ.

3.2.2 Animals and management

Following a 14-day baseline measurement period (14 February – 27 February 2018), where all cows grazed RGWC herbage, 54 mid-lactating Friesian x Jersey dairy cows were stratified into nine groups of six cows, according to milk yield (19.0 ± 1.17 kg/day), milk solids production (1.72 ± 0.01 kg MS/cow.day), days in milk (168 ± 8.9 days), and live weight (484 ± 20.0 kg) (means ± SEM) and randomly assigned to replicated paddocks (n = 3) of chicory, plantain or RGWC. Rumensin Anti-Bloat Capsule (Elanco Animal Health, Auckland, New Zealand) was orally administered to all cows seven days before the experiment. From 28 February to 6 March, cows were adapted to their forage treatments by increasing the relative proportion of herb in the diet. The measurement period took place over nine days between 7 and 15 March.

3.2.3 Animals and management

Following a 14 day baseline measurement period (14 February – 27 February 2018), where all cows grazed RGWC herbage, 54 mid-lactating Friesian x Jersey dairy cows were stratified into nine groups of six cows, according to milk yield (19.0 ± 1.17 kg/day), milk solids production (1.72 ± 0.01 kg MS/cow.day), days in milk (168 ± 8.9 days), and live weight (484 ± 20.0 kg) (means ± SEM) and randomly assigned to replicated paddocks (n=3) of chicory, plantain or RGWC. Rumensin Anti-Bloat Capsule (Elanco Animal Health, Auckland, New Zealand) was orally administered to all cows seven days before the experiment. The experiment took place over 21 days which included an adaptation period (7 days) in which cows were adapted to their forage treatments by increasing the relative proportion of herb in the diet, urine measurement period (9 days,) and a milk fatty acid composition measurement period (5 days).

3.2.4 Herbage measurements

Target allowance was 30 kg/cow.day DM above ground level to maintain baseline milk production. A new allocation was offered daily after the morning milking. Temporary fencing was used to control allocated areas and free access to fresh water was always available . Herbage dry matter allocation for all replicate groups was based on pre-graze herbage mass determined from quadrat cuts. Quadrat cuts were harvested pre- grazing on days 8, 11 and 14 by cutting all herbage within a 0.25 m2 quadrat to ground level using electric hand shears. The procedure was repeated the following day in the immediately grazed area to determine post-graze mass. The harvested herbage within each quadrat was washed and oven dried at 60 °C to a constant weight.

Herbage samples for chemical and botanical composition analysis were collected to ground level, pre and post grazing at 0630 h on day 8, 11, and 14 of the experiment by harvesting at random locations within a paddock. Each sample was transported to the lab for immediate processing.

Herbage was homogenised and sub-sampled for botanical and chemical analysis. For botanical composition, the sub-sample was dissected into sown species, weeds, and dead material. The sorted components were dried at 60 °C until they reached constant weight. Moisture content of the sub sample was determined by recording fresh weight (approximately 40–60 g) and dry weight following oven drying. The sample for chemical analysis was immediately frozen at −20 °C, freeze-dried and ground to pass through a 1-mm sieve (ZM200 Retsch). Dried ground samples were analysed for organic matter (OM), water soluble carbohydrates (WSC), neutral and acid detergent fibre (NDF, ADF), crude protein (CP), dry matter digestibility (DMD), organic matter digestibility (OMD) and digestible organic matter in the dry matter (DOMD) by near infrared spectrophotometry (NIRS, Model: FOSS NIRSystems 5000, Maryland, USA). Calibration equations for crude protein (Variomax CN Analyser, Elementar), WSC (MAFF, 1986), NDF (Van Soest et al., 1991) and ADF (method 973.18; AOAC, 1990), DOMD and DMD (Iowerth et al., 1975) were previously derived on RGWC, chicory and plantain forages. The R-squares for predicting CP, OM, WSC NDF, ADF, DMD, DOMD and OMD for both herbs and RGWC were similar and were all above 0.9. All samples were well within the calibration range.

All water troughs were fitted with flow meters to measure group water intakes. Apparent dry matter intake (DMI) was determined on day 8, 11 and 14 from the difference between pre- and post-grazed herbage mass and the area grazed i.e. dry matter intake (kg/ cow.day DM) = {[(pre kg/ha DM – post kg/ha DM) ÷ no. of cows] x area. Apparent nutrient intake (nitrogen and minerals) was determined using the same equation as DM intake but multiplying herbage mass by the herbage nutrient concentration in the mass pre and post-grazing.

3.2.5 Animal measurements

Milk yield was measured twice daily (0700 and 1430 h) with an automated system (DeLaval Alpro Herd Management System, DeLaval, Tumba, Sweden). Two milk sub-samples were collected from individual cows during the morning and afternoon milking of days 8, 11, and 14. Milk samples were analysed for fat, protein, and lactose content (Livestock Improvement Corporation Ltd., Christchurch, New Zealand) using Milkoscan™ (Foss Electric, Denmark). To determine milk urea nitrogen (**MUN**), milk samples were centrifuged at 4000 g for 10 min at room temperature and refrigerated for 10 min to allow the fat to solidify on the top and be removed. Milk urea nitrogen was determined on skimmed milk by an automated Modular P analyser (Roche/Hitachi; (Talke and Schubert, 1965).

Grazing behaviour was determined on three focal animals in each group which were fitted with SensOor eartags (Agis Ltd, the Netherlands) for continuous recording of grazing and ruminating time. Total grazing, ruminating and idle time were summed separately for each animal within each mob.

Urine and faecal analyses were carried out on spot samples collected after the morning and afternoon milking on day 14 from all animals. Urine was collected by valval stimulation, while faeces were sampled via rectal grab. Urine samples were immediately acidified with sulfuric acid to prevent volatilisation of N. Faecal samples were lyophilized and ground to pass 1-mm sieve. Mineral composition for herbage and urine samples was determined by inductively coupled plasma atomic emission spectroscopy (Koons, 2003). Urine N and Faecal N concentrations were determined by combustion (Vario MAX CN, Elementar Analysensysteme, Hanau, Germany). Urine urea content was determined using a commercial enzymatic kinetic technique (Randox, Crumlin, Co., Antrim, UK). Creatinine concentration was determined by the Jaffe method (Daytona RX Clinical Analyser, Randox, Nishinomiya, Japan).

3.2.6 Urination behaviour

Urination behaviour was measured using 'Lincoln University PEETER sensors' which had been developed and validated prior to the experiment. Briefly the 225 g sensor includes a micro-processor (Feather M0 Proto, Adafruit P2772), pressure sensor (Honeywell 1PSI Diff 3.3v HSCMRRN001PDAA3, Digi-Key 800 344–4539), time clock, SD memory card and battery (LiPo 800 mA h) encased in a sealed polytube sleeve. The PEETER sensor works on the principle of pressure differentiation, in this case the majority of differential pressure comes from height of fluid acting upon the pressure sensor inlet orifice. Urine volume (V) is calculated based on the modified Bernoulli's equations; V=bxEXP where b is CV (and CV = 2 gH, where g = acceleration of gravity H =height (m) derived from the flowtime), x is the Pascal's reading followed by the resulting exponent. For all the sensors, laboratory validation for sensor reading and water volume showed goodness of fit (R2 > 0.99), with accuracy of prediction improving at volumes greater than one litre (< 0.75 L ± 15%, and >1 L ± 2.5%, Figure 3.1).

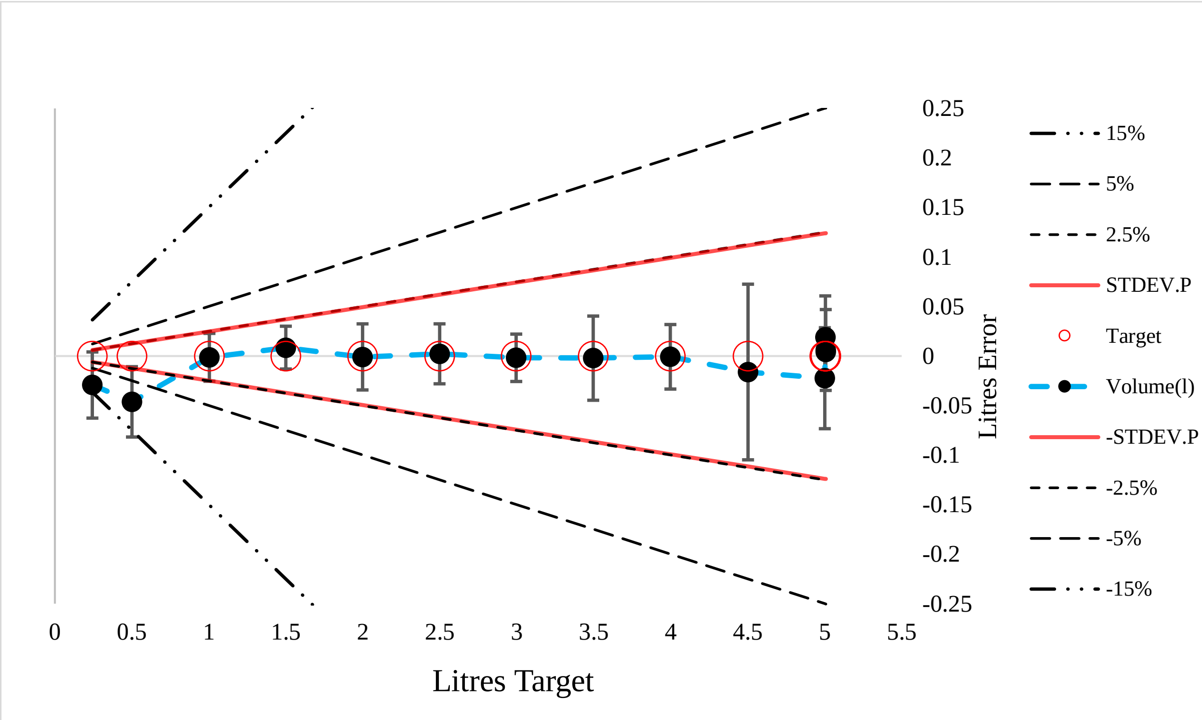

Figure 3.1. Calibration evaluation for the sensors used in the study (average for all sensors used in the study.

A sensor sleeve was attached over the vulva of the cow using a ventilated 3D printed mould secured by a biocompatible glue (Plate 3.2). The 3D printed mould was constructed from acrylonitrile butadiene styrene and printed using an UP-Mini 3D Printer (3D printing Systems, Auckland, NZ). The total sensor unit weight was less than 400 g.

A balanced incomplete block design was utilized which incorporated three runs in which four cows per treatments wore the sensor. Selection was done in such a way that at least one cow within a group wore the sensor. Run 1 compared plantain and chicory (days 1–4), Run 2 compared plantain and RGWC (days 4–6), and Run 3 compared chicory and RGWC (day 6–9). The urine sensors were attached after milking and remained on the cows for 48 h. To minimise yard time and disruption of natural animal behaviour eight sensors were deployed on any given day during the nine-day measurement period. The sensors were monitored during each milking, SD cards were downloaded, and rechargeable batteries were replaced every 24 h. Cows were kept in the yard during this time (˜ 30 min). If a sensor showed any sign of detaching, a new harness was attached to another cow in the same treatment during the same run. As a result, data was collected from a total of thirty-five cows; eight cows on chicory and seven cows on plantain during run one; four cows on RGWC and four cows on plantain during run two; seven cows on chicory and five cows on RGWC during run three. A complete set of 24-h data was collected from 30 cows, and data for two consecutive days was collected from 10 of the 30 cows (40 observations).

Plate 3.2**.** A sensor sleeve attached over the vulva of the cow using a ventilated 3D printed mould secured by a biocompatible glue.

3.2.7 Statistical analysis

For all data, paddock was the experimental unit. Forages, milk production, urine and fecal spot samples, and intake measurements were compared using a mixed model ANOVA in R using the 'nlme' package (Pinheiro et al., 2018). All models included treatment as the fixed effect. For data taken on individual animal (milk production, urine and fecal spot samples) animal nested in sampling day was used as a random effect. For data taken at the paddock level (diet quality, water and forage intake) sampling day was used as a random effect. Means separation were done using the 'emmeans' package of R, with Tukey's method for comparing a family of 3 estimates, and the default degrees-of-freedom approximation, containment. A significant difference was declared at $P < 0.05$, while a tendency was declared at $P < 0.10$.

Urine parameters were analysed separately. The urine harness occasionally became unstuck and lying/standing periodically triggered a recorded event. To manage non-urine event data, any consecutive data with short time intervals (< 5 min), or short duration (< 5 s), were excluded, similarly events with a flat pressure curve were also excluded (Edwards et al., 2015; Ravera et al., 2015; Shepherd et al., 2017). For daily urination patterns (urination events/cow.day, time between urination events, urine volume/cow.day, and urine volume/event) only observations from cows with a full 24 h dataset was used (40 observations). Run did not significantly influence urination patterns ($P > 0.10$), hence a balanced incomplete block design was used to compare means across 'Runs'. For analysis, data were averaged across days and animals within a replicate and paddock was used as an experimental unit. The 'aov.ibd' function in the 'ibd' package was used to conduct this analysis in R (R Core Team, 2018, v.3.4.4). Tukey's HSD was used for mean separation. For the appropriateness of using ANOVA to analyze urination harness data, normality and homoscedasticity of errors was checked using Shapiro-Wilk's test and Bartlett's test, respectively. Data was found to be normally distributed ($P \geq 0.13$) and to have homoscedastic errors ($P \geq 0.22$). Pearson's correlations between urination patterns and forage characteristics were generated using the 'cor' function in base R (R Core Team, 2018, v.3.4.4).

To explore treatment effects on diurnal variation of urination patterns (urine volume and time between urinations), fixed effects regression models in R (R Core Team, 2018, v.3.4.4) were fit to each individual urination event. The independent variable used was hour of the day. Linear, quadratic, and cubic terms were evaluated and the highest significant order term was reported upon significance ($P \leq 0.05$). Mean centered initial body weight was explored as a covariate for the linear regression analysis and explained a significant amount of variation ($P \leq 0.02$). Body weight was mean centered so that when the regression of hour of day on the dependent variables was graphed the illustration would represent the average body weight of a cow. Forage type effects on diurnal variations were determined by the significant interaction of treatment and the highest order term.

In order to explore the hourly grazing and ruminating behaviour for each forage type, a fixed model was fit which included treatment, hour of the day, and forage type x hour of the day interaction as fixed effects. Least-square means were generated and upon statistical significance of the ANOVA, treatment mean separation was done using Tukey's HSD from the 'lsmeans' package. Data was processed and figures were generated using the 'tidyverse' and 'ggplot2' packages, respectively, in R (R Core Team, 2018, v.3.4.4).

3.3 Results

The minimum temperature ranged between 5.8–15.8 °C (average 10.2 °C) and the maximum temperature ranged between 14.8–24.3 °C (average 19.7 °C) during the experiment. Sunrise occurred between 0715 and 0726 and sunset occurred between 1949 and 2003 h. The conditions were dry except 6.8 mm and 3.0 mm rain on respective days 8 and 11 of the experiment.

3.3.1 Herbage characteristics

Post establishment growth rate of plantain had been slow compared with chicory and RGWC, so this treatment area was given five-week regrowth instead of three-week regrowth as for RGWC and chicory. However, growth rate of plantain increased during the setup phase resulting in greater pre-graze herbage mass after a longer regrowth interval (Table 3.1). Both chicory and RGWC pastures were in a vegetative state while plantain contained 210 g/kg DM as seed head. Chicory and plantain pastures had less than 50 g/kgDM white clover, while RGWC swards consisted of 131 g/kgDM white clover. In plantain and RGWC, weed content was 103 and 63, while dead material was 71 and 60 g/kgDM, respectively. Weed content and dead material were both less than 50 g/kgDM of DM in chicory. Seed head in plantain pastures increased to 300 g/kg of total DM after grazing.

Plantain and chicory had lower DM, OM, and fibre contents than RGWC (Table 3.1, $P < 0.01$). The seed head in plantain herbage resulted in lower CP content than chicory and RGWC herbage. Chicory herbage was more digestible than plantain or RGWC ($P < 0.05$). Non-fibre carbohydrates were 2.1 and 1.9 times higher in chicory and plantain than RGWC ($P < 0.01$), respectively. The three predominant minerals in the herbages were Sodium (Na), potassium (K) and calcium (Ca), which were greater (P < 0.05) in herbs than RGWC. For cows grazing chicory, plantain or RGWC, there were differences (P < 0.05) in mineral intake with respective apparent intake for Na were 208, 130 and 23.4 ± 3.1 g/cow.day and for K were 635, 447 and 341 ± 5.01 g/cow.day.

Table 3.1. Herbage mass and pre-grazing chemical composition of chicory, plantain and ryegrass/white clover (RGWC) sampled to ground level.

Item	Chicory	Plantain	RGWC	SEM	P-value
Pre graze mass (kg/ha DM)	2711^{c}	4609^{a}	3097^{b}	116	<0.01
Post graze mass (kg/ha DM)	791^{c}	1435^{b}	1759^{a}	69	<0.01
Dry matter (g/kg FW)	81^{c}	105^{b}	151^{a}	2.8	<0.01
Organic matter (g/kg DM)	863^{b}	863^{b}	911^{a}	3.2	<0.01
Crude fat (g/kg DM)	39^{b}	32^{c}	46^{a}	1.9	<0.01
Crude protein (g/kg DM)	195^{b}	137^{c}	218^{a}	7.9	<0.01
NDF (g/kg DM)	204^{c}	310^{b}	444^{a}	6.3	<0.01
ADF (g/kg DM)	174^{c}	221^{b}	249^{a}	4.2	<0.01
WSC[1] (g/kg DM)	128^{a}	52^{c}	114^{a}	8.2	<0.01
NFC[2] (g/kg DM)	426^{a}	384^{b}	202^{c}	6.8	<0.01
ME (MJ/kg DM)	12.3^{a}	11.5^{c}	11.9^{b}	0.08	<0.01
DOMD (g/kg DM)	768^{a}	721^{c}	746^{b}	4.8	<0.01
DMD (g/kg DM)	848.3^{a}	770.5^{b}	771.1^{b}	5.3	<0.01
Calcium (g/kg DM)	9.58^{b}	18.2^{a}	5.81^{c}	0.43	<0.01
Magnesium (g/kg DM)	2.55^{a}	1.03^{c}	1.83^{b}	0.11	<0.01
Phosphorus (g/kg DM)	4.15	3.73	3.58	0.17	0.06
Potassium (g/kg DM)	36.6^{a}	27.7^{b}	19.7^{c}	0.23	<0.01
Sodium (g/kg DM)	12.1^{a}	7.5^{b}	1.5^{c}	0.78	<0.01
Sulphur (g/kg DM)	4.25	3.76	3.48	0.24	0.10

$^{a–c}$ Means within a row with different superscripts differ (P < 0.05).

[1] Water soluble carbohydrates.

[3]Non-fibre carbohydrates = (1000- (g/kg DM NDF + g/kg DM CP + g/kg DM Fat + Ash))

3.3.2 Dry matter intake, milk production and composition

Because of variation in estimated pre-graze herbage mass, the mean area allocated each day was 109, 62 and 101 ± 3.7 m^2/cow/ day (P < 0.001) for chicory, plantain and RGWC respectively. This resulted in actual allocations, above ground level, of 26 ± 1.8, 27 ± 2.2 and 32 ± 2.4, kg/cow.day, for cows offered chicory, plantain and ryegrass respectively (P < 0.05). Based on pre- and post-grazing quadrats, cows grazing the herbs utilised 66% of the allocated herbage, while those grazing RGWC utilised 46%. Apparent DMI was greater for herbs than RGWC (Table 3.2: P < 0.01). Milk yield and milk solids produced (protein + fat) were higher in cows grazing chicory than RGWC

(Table 3.2: P < 0.01). Cows grazing plantain produced intermediate milk and milk solids yield and were similar to cows grazing RGWC (P > 0.05). Milk fat content tended to be greater for cows grazing chicory and RGWC than plantain (P = 0.09). Milk fat yield was greater for cows grazing chicory than for cows grazing plantain or RGWC (P<0.01).

Table 3.2. Apparent dry matter intake (kg/cow.day of DM), milk yield and milk composition from cows fed chicory, plantain and ryegrass/white clover (RGWC).

	Chicory	Plantain	RGWC	SEM	P-value
Apparent dry matter intake	17.3^{a}	17.4^{a}	16.2^{b}	0.75	<0.01
Milk yield (kg/cow.day)	19.8^{a}	19.3^{ab}	18.2^{b}	0.40	<0.01
Milk solids (kg/cow.day)	1.86^{a}	1.76^{b}	1.72^{b}	0.03	<0.01
Fat (g/100 g milk)	5.42	5.10	5.36	0.11	0.09
Protein (g/100 g milk)	4.09	4.06	4.19	0.05	0.21
Lactose (g/100 g milk)	5.01	5.04	5.01	0.03	0.42
Fat yield (kg/cow.day)	1.06^{a}	0.98^{b}	0.96^{b}	0.02	<0.01
Protein yield (kg/cow.day)	0.80^{a}	0.78^{ab}	0.76^{b}	0.01	0.02
Lactose yield (kg/cow.day)	0.99^{a}	0.97^{a}	0.91^{b}	0.02	<0.01

$^{a–b}$ Means within a row with different superscripts differ (P < 0.05).

3.3.3 Grazing behaviour

Average time spent grazing was similar between cows grazing chicory and plantain (593 ± 15.4 min per cow per day), but greater than cows grazing RGWC (379 ± 15.4 min per cow per day; P < 0.001). There were two major grazing bouts per day i.e. between AM and PM milkings and after PM milking (Figure 3.2). Grazing stopped when cows were going to the shed or around 1900 h (sunset). Cows grazing chicory, plantain, or RGWC ruminated for 143, 335 or 420 ± 13.9 min/day, respectively (P < 0.01). Rumination occurred mainly between 2000 h and 0600 h, where nearly 30 min per hour was spent in this activity for those cows on the plantain and RGWC treatment. By contrast only 10 min per hour was spent ruminating by cows on the chicory treatment (Figure 3.2). During this time, cows grazing chicory spent most of their time idle (P < 0.05).

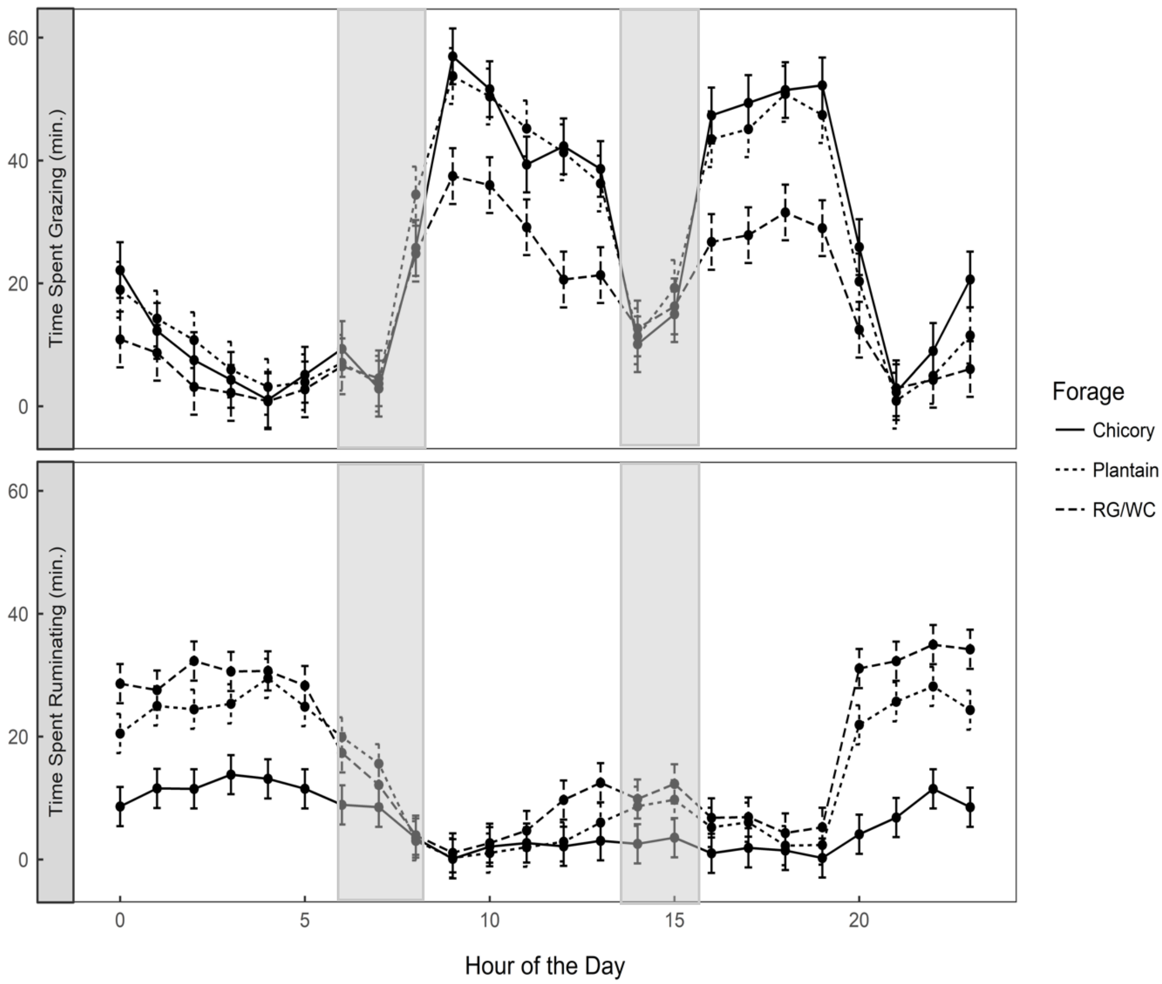

Figure 3.2. Diurnal variation of time spent grazing and ruminating as influenced by forage type. Chicory, plantain and ryegrass/white clover treatments are represented by the, long dashed, short dashed and solid lines, respectively. Shaded areas represent AM and PM milking events. Points are LSMEAN for each forage type at each hour and error bars are standard error of the mean.

3.3.4 Water balance

Cows grazing RGWC drank more trough water than cows grazing chicory or plantain (Table 3.3; $P < 0.05$). Total water intake was greater in cows grazing herbs than cows grazing RGWC ($P < 0.05$), which was driven by feed water intake which accounted for 96%, 92%, and 79% of total water intake for cows grazing chicory, plantain and RGWC, respectively. Mean water excreted through milk was highest from cows grazing chicory, intermediate for those grazing plantain and lowest for those grazing RGWC ($P < 0.01$). Cows grazing chicory excreted 1.5- and 2.4-times greater urine volume per day ($P < 0.01$) than cows grazing plantain and RGWC, respectively.

Cows grazing plantain excreted an intermediate urine volume per day and was 1.6 times greater than RGWC (P < 0.05).

Table 3.3**.** Means of variables used for estimating water balance in cows grazing chicory, plantain and ryegrass/white clover (RGWC).

Item	**Chicory**	**Plantain**	**RGWC**	**SEM**	**P-value**
Water intake (L/cow.day)					
From troughs	7.2^{c}	12.5^{b}	25.8^{a}	0.76	<0.01
From herbage	195.9^{a}	139.5^{b}	97.5^{c}	3.46	<0.01
Total water intake (L/cow.day)	203.1^{a}	152.0^{b}	123.4^{c}	3.91	<0.01
Water in milk (L/cow.day)	17.9^{a}	17.5^{ab}	16.4^{b}	0.37	<0.01
Total Urine Volume (L/cow.day)	100.5 ± 4.0^{a}	67.8 ± 5.3^{b}	42.2 ± 5.4^{c}		<0.01
Faeces DM (g/kg DM)	153.4^{a}	157.5^{a}	117.6^{b}	7.28	0.02
Faeces Ash (g/kg DM)	297.4^{a}	224.1^{b}	234.2^{b}	8.60	<0.01

$^{a–c}$ Means within a row with different superscripts differ (P < 0.05).

3.3.5 Urination behaviour

The total number of urination events recorded from the 35 cows used during the experiment was 1034. The average time between urination events was 101 ± 4.5, 65 ± 5.7 and 51 ± 3.2 min for cows grazing RGWC, plantain, and chicory, respectively (P < 0.05). Cows grazing chicory and plantain urinated 28.6 ± 1.1 and 21.2 ± 1.5 times per day, respectively (P > 0.05), while those grazing RGWC urinated 13.9 ± 1.5 times per day which is significantly less than the herbs (P < 0.05). There was large variation in urine event volume (0.13–11.6 L/event), though mean urine volume per event was similar for each treatment (3.01 ± 0.3 L/event; P= 0.24).

There was a significant cubic relationship between urination frequency or urination volume and time (P < 0.05), though the goodness of fit was low (R^2 = 0.32 and 0.18, respectively). The fitted model included mean centered initial body weight, so Figure 3.3 represent the regression lines for each treatment for the average herd body weight. The time between urination events was consistently greater throughout the day in cows grazing RGWC than chicory and plantain (Figure 3.3). More urination events were recorded during PM grazing (3:30 – 8:30 PM). Consequently, the time between urinations was lowest during PM grazing. There was a significant interaction (P < 0.01) between the hour of the day coefficients for plantain and chicory for time between events.

The time between urinations in cows grazing chicory was lower than plantain for the larger proportion of the day (P < 0.05), but similar when the cows were gathered for PM milking, during milking and PM grazing (c. 1:30 PM - 8:30 PM; P > 0.05). The diurnal pattern of urine volume per urination event is shown in Figure 3. The urine volume per event was more variable during the night.

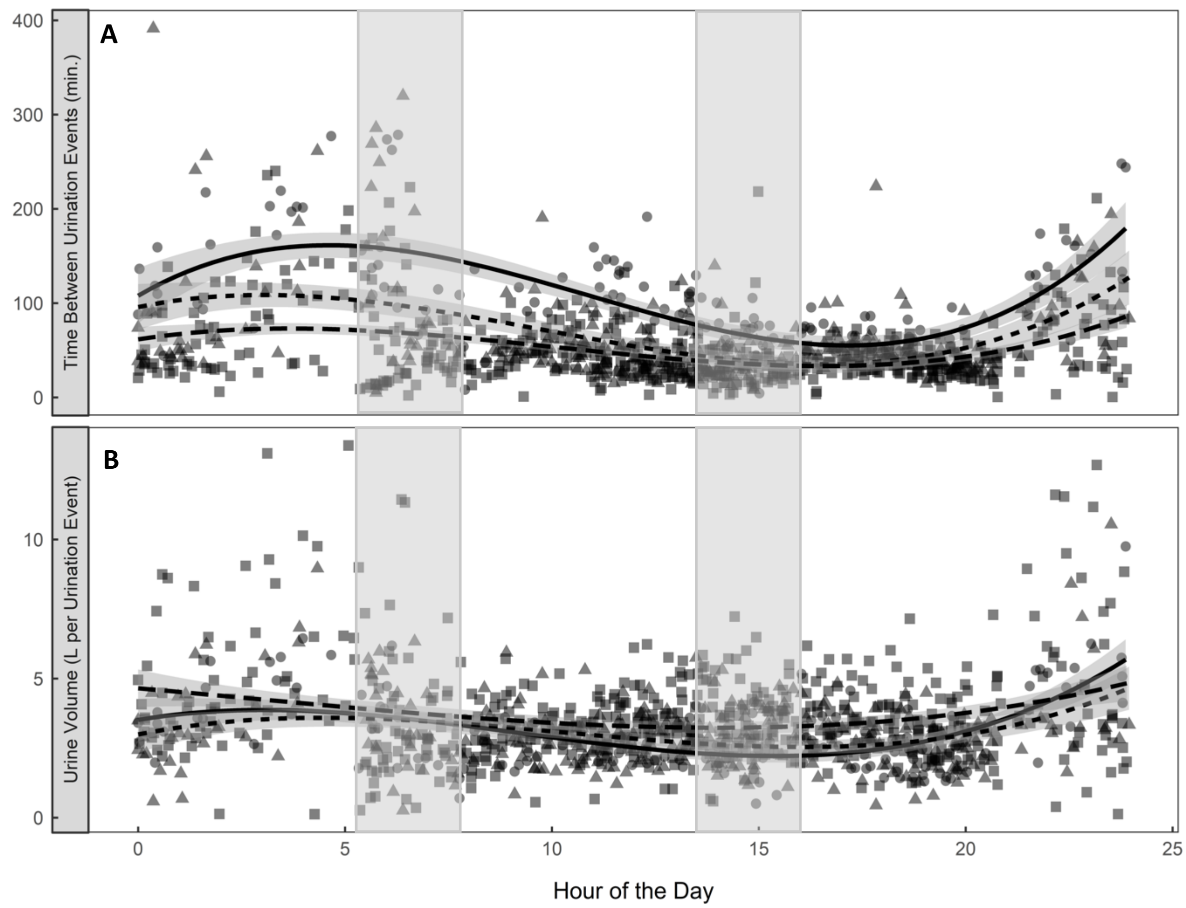

Figure 3.3. Diurnal variation of (A) time between urination events and (B) urine volume as influenced by forage type. Square points and long dashed line points denote chicory, triangle points and short dashed line denote plantain and, circle points and solid line denote ryegrass/white clover. Each point represents a single urination event. Shaded areas represent AM and PM milking events.

3.3.6 Nitrogen utilisation

Apparent N intake was similar for cows grazing chicory and RGWC (P > 0.05), but greater than for those grazing plantain (Table 3.4; P < 0.01). Nitrogen use efficiency for milk (g N in milk/g N eaten) was 0.24, 0.29 and 0.21 for cows fed chicory, plantain and RGWC respectively. Milk urea nitrogen was greatest in cows grazing RGWC, intermediate for those grazing chicory, and lowest for those grazing plantain ($P < 0.05$). Urine urea was highest for cows grazing RGWC, but similar for cows grazing the herbs. Urine N concentration and NH_3 were also similar for cows grazing chicory and plantain ($P > 0.05$), and higher for RGWC ($P < 0.05$). There was greater faecal moisture in spot samples from cows fed RGWC compared with cows fed herbs. Faecal N was similar for all treatments ($P = 0.99$).

Table 3.4. Apparent N intake, urine composition and faecal N concentration from cows grazing chicory, plantain and ryegrass/white clover (RGWC).

	Chicory	**Plantain**	**RGWC**	**SEM**	**P-value**
Apparent N intake (g/cow.day)	520^{a}	420^{b}	566^{a}	18.3	<.001
Milk Urea (mmol/L)	3.25^{b}	2.36^{c}	4.92^{a}	0.33	<.001
Milk Urea N (mmol/L)	6.50^{b}	4.71^{c}	9.83^{a}	0.66	<.001
Milk N output (g/cow. day)	125.7^{a}	120.8^{ab}	118.3^{b}	1.23	0.02
Urine N (g/kg)	1.28^{b}	1.28^{b}	4.83^{a}	0.43	<.001
Urine Urea (mmol/L)	29.2^{b}	27.1^{b}	128.7^{a}	14.5	<.001
Urine NH_3 (mmol/L)	0.37^{b}	0.40^{b}	0.67^{a}	0.19	<0.01
Creatinine (mmol/L)	0.59^{b}	0.80^{b}	1.44^{a}	0.06	<.001
Urine calcium (g/kg)	0.03	0.03	0.04	0.02	0.62
Urine magnesium (g/kg)	0.02^{b}	0.02^{b}	0.05^{a}	0.008	<0.01
Urine phosphorus (g/kg)	0.01	0.01	0.01	0.002	0.98
Urine potassium (g/kg)	4.25^{b}	3.54^{b}	9.72^{a}	2.3	<.001
Urine sodium (g/kg)	1.23^{a}	0.92^{b}	0.32^{c}	0.16	0.01
Urine sulphur (g/kg)	3.12	2.38	2.97	0.40	0.38
Feces N (g/kg)	32.4	32.3	32.4	0.08	0.99

$^{a–c}$ Means within a row with different superscripts differ ($P < 0.05$).

3.3.7 Urine purine derivatives and mineral concentration

Urine creatinine concentration was 1.8 and 2.4 times higher ($P < 0.05$) in cows grazing RGWC than plantain and chicory, respectively. Urinary mineral concentrations of phosphorus and Ca were less than 0.05 g/kg and did not differ between forage diets. The concentration of Na in spot urine samples reflected concentrations in the herbage but spot urine K concentrations were greater in urine of cows grazing RGWC.

3.4 Discussion

The main hypothesis of the current research was that forage type would alter urination patterns of dairy cows without altering their productivity. This was based on current understanding of the impact of nutritional, biochemical, and morphological properties of forages, on ingestive behaviour, and water excretion of dairy cows. The data collected in this study supports hypotheses on changes in urine excretion due to forage type.

3.4.1 Dry matter intake and milk production

In spite of the slightly lower DM allocation to the herb groups, compared with RGWC, apparent DMI was greater for cows grazing herbs compared with those grazing RGWC. This was due to greater utilisation of herbs which had low bulk density at the base of the sward, enabling animals to consume more DM of available herbage Plate 3.3. Interestingly cows offered chicory were able to improve milk yield and milk solids, while those on RGWC maintained similar milk production as was achieved during the covariate period (1.72 kg MS/cow.day). The increase in milk production from cows grazing sole diets of chicory is consistent with earlier findings from cows fed diets containing proportions of chicory (Chapman et al., 2008; Minneé et al., 2017) and it reflects the greater DMI intake reported in the current study. The lower DMI observed in the current study for cows grazing RGWC maybe a result of greater fibre content compared with chicory (444 vs 204 g/kg DM). Intake was likely reduced as a result of reduced grazing time as cows compensated grazing for ruminating time in order to break down the fibre (Figure 3.2A).

Metabolisable energy and non-fibre carbohydrates were also high in chicory than RGWC. Minneé et al. (2012) also reported greater milk yield in cows grazing chicory when metabolisable energy was higher than RGWC. Cows grazing plantain, on the other hand, produced similar milk solids to RGWC, despite tending to have greater intakes than RGWC. In previous experiments, milk yield of cows grazing pure (Box et al., 2017a), or mixed (Totty et al., 2013), plantain pastures were similar or greater than those grazing traditional RGWC pastures. In the current study, we expected plantain and chicory to have similar milk yield which would be greater than RGWC. However, because the plantain pasture was grazed at a more advanced age, resulting in considerable amount of seed head which decreased CP and increased fibre content, we did not observe increased milk yield relative to the control RGWC.

Plate 3.3. Chicory and ryegrass/white clover bulk density at the base of the sward post grazing.

3.4.2 Urination behaviour

This is the first study to investigate the urinating pattern of dairy cows grazing chicory and comparing them to plantain and RGWC. Urine excretion in ruminants is largely governed by water intake and excretion of minerals such as Na and K (Ledgard et al., 2009; Spek et al., 2012). While we acknowledge the relatively short observation period of this study, the urination patterns reported here are in line with previous research, providing sufficient evidence on which to formulate further hypotheses.

3.4.2.1 Urine volumes

Our results show substantial variation in urination between cows grazing either of the three forages used in the study. Large variation in volume and frequency of events has been previously reported (Betteridge et al., 2013; Selbie et al., 2015; Bryant et al., 2018) using an alternative sensor. Compared with this study the mean urine volume from cows grazing RGWC reported in those studies were similar to cows in late lactation in Canterbury (Bryant et al., 2018) but greater than cows in the Waikato (Betteridge et al., 2013; Shepherd et al., 2017).

We hypothesised that feed water intake from herbs would be large and lead to greater urinary output from cows grazing those forages than from cows grazing RGWC. This was confirmed by Pearson's correlation analysis which revealed that feed water intake in this study was highly correlated with urine volume per day ($R = 0.81$; $P < 0.05$). Dry matter content of the herbs was low, and cows grazing the herbs spent most of their time grazing, presumably to meet their nutritional requirements. As a result, cows grazing herbs ingested more water per day than cows grazing RGWC. Sodium, K and N intakes have been suggested as other explanatory variables for urine production in ruminants (Nennich et al., 2006; Dijkstra et al., 2013). Indeed, Na and K intakes were positively correlated to urine volume per day ($R = 0.65$ and 0.70, respectively; $P < 0.01$). The intakes of Na and K were 8.7 times and 1.8 times higher in cows grazing chicory than RGWC, respectively, and 5.4 times and 1.3 times higher in cows grazing plantain than RGWC. Potassium intake was 29% and 46% greater in chicory than plantain and RGWC herbage, respectively. De Campeneere et al. (2006) also reported 2.4 times greater urine production in animals fed grass silage diet rich in Na and K than maize silage-based diets with much lower Na and K. It is, therefore, not surprising that cows grazing chicory excreted 1.6- and 2.4-times greater urine volume per day than cows grazing plantain and RGWC, respectively. Contrary to previous studies, apparent N intake in this study was not related to urine volume per day ($R = 0.02$; $P > 0.05$).

3.4.2.2 Urine distribution

In this study we found that by feeding alternate forages there was little change to the mean volume per event, instead frequency of urination was increased from those cows with greater urine volume. Consequently, it was urination frequency rather than urine volume per event which dictated total daily urine volume. Other studies have looked at urination behaviour either by using sensors or observing animals. In a review, Selbie et al. (2015) summarised several studies which revealed that cows grazing RGWC urinate 9–14 times per day. Shepherd et al. (2017) later also reported an average of 14.1 events/day for dairy cows grazing RGWC. These findings are similar to the 13.9 events/day for cows grazing RGWC in the current experiment.

There was large variation in urine event volume. The volume per event for cows grazing RGWC ranged between 0.51–9.75 L/event, which is near the range of 0.9–20 L per event reported by Selbie et al. (2015). Likewise, the time between urination events for RGWC (101 min) reported in the study falls well between the 98 min reported by Shepherd et al. (2017) and the 111 min reported by Bryant et al. (2018).

Our data fit the expected natural circadian rhythms of urination behaviour, with evidence of increased bladder storage during the night (Noh et al., 2011) as observed in the high volume events in the morning and more frequent smaller events during the day (Figure 3B). The frequency of urine events increased when cows were gathered for PM milking and during milking which was expected (Shepherd et al., 2017), and is associated with high activity. Similar findings by (Aland et al., 2002), who observed more urination activities (reduction in time between urination events) when cows were most active (during feeding and milking), further corroborate the current experiment`s observations. Additionally, more frequent urinations were recorded during PM grazing (1530 h – 2030 h) than during AM grazing (0830 h – 1330 h), in line with observations made by Draganova et al. (2016). Coincidentally, cows also spent more time grazing during PM grazing than during AM grazing (Figure 3.2A). A smaller secondary peak in urination events occurred between 2300 h and 0000 h, which also coincided with a small peak of grazing intensity at 2300 h (Figure 3.2A). Our results suggest that cows urinate more frequently when they spend more time grazing: the three peaks of grazing observed in this study (i.e. 1000 h–1300 h, 1600 h–2000 h and 2300 h to 0000 h) corresponded well with three peaks of urination events. The pattern is the same in all three forages, but the intensity of grazing is higher for cows grazing the herbs than RGWC, which appeared to impact the density of urination events.

3.4.3 Nitrogen utilisation

A secondary objective of the study was to compare the effects of the three forages on nitrogen utilisation. Previous studies (Shepherd et al., 2017; Bryant et al., 2018) have used refractometers to report diurnal variation in UN concentration, because we were only able to measure spot UN concentration at two time points our conclusions around UN excretion are conservative. Results from urine spot samples revealed that UN concentration in cows grazing RGWC was 3.7 times higher than cows grazing chicory or plantain, but well within the general range of spot UN concentration reported in literature (Bryant et al., 2017; Cheng et al., 2017). Apparent N intake was similar between cows grazing chicory and RGWC but more N was partitioned to milk from cows fed chicory than RGWC (24 compared with 20% of N consumed, Table 4). In this study feeding plantain, albeit at a more mature stage of growth than the other forages, resulted in the greatest dietary N conversion to milk N (28% of N consumed), this was largely as a result of lower N intake. The N concentration of the dung was similar for all treatments, so again due to the moderate digestibility of plantain, the proportion of N excreted in the dung would likely be relatively high compared with RGWC or chicory. Thus, we anticipate that optimal N partitioning from an environmental perspective, likely occurred from cows fed plantain as intake N was low and a higher proportion of intake N was estimated to have been partitioned to milk and dung (60%) compared with RGWC or chicory (only 40% N in milk and dung). However, while total UN excretion from cows grazing chicory or RGWC is likely to be similar, we argue that feeding chicory dilutes urinary N and reduces soil N loading under the urine patch.

The implications of these findings indicate the potential to reduce nitrate leaching from alternative forages. Nitrate leaching risk from urine events is influenced by N load which is a function of volume and concentration of N (Li et al., 2012). These results demonstrated that forages may be able to alter N leaching risk by altering the distribution of UN excretion to reduce N load. In this instance, cows grazing chicory or plantain urinated more often and lowered UN concentration compared with those grazing RGWC. At the urine patch level this is certainly expected to reduce nitrate leaching, though extrapolation to total urine patch area coverage as a result of more urine patches may, at the paddock scale, off-set immediate benefits in reduced N leaching. Cows grazing the herbs have demonstrated the potential to reduce environmental risks, however, given the limited practicality of having farms entirely sown with the herbs, further studies are recommended to determine suitable ways to integrate the forage herbs into the dairy farming systems, while sustaining the effects on urination behaviour.

3.5 Conclusions

Forage herbs with their high water and mineral contents demonstrated potential to minimize environmental risks significantly by increasing urinating frequency and substantially lowering urinary nitrogen concentration. This was achieved while maintaining, or in the case of chicory, improving milk solids production. Further work is required to confirm the results and to measure the total daily UN deposited onto pasture from cows grazing chicory and plantain.

Chapter 4

4. Grazed chicory, plantain or ryegrass–white clover alters milk yield and fatty acid composition of late-lactating dairy cows

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4.1 Introduction

Chicory (*Cichorium intybus* L.) is a deep-rooted, summer-active perennial herb that has been listed as having a high feeding value (Waghorn et al., 2007). Despite the beneficial qualities of chicory, producers rarely integrate chicory into their dairy farming systems. This is partly because of the limited information available on dairy cows fed chicory, either as a monoculture or a mix with ryegrass–white clover swards. Plantain (*Plantago lanceolata* L.) is also a perennial herb that is gaining popularity with dairy farmers due to its ability to support high milk yield and dilute urinary nitrogen (Box et al., 2017a) for environmental gains. Integration of novel forages into farm systems is being considered for their potentially beneficial effects on the environment. Given the increasing interest in alternative forages, more information is required on product quality of cows fed those diets.

Processors of dairy products have indicated interest in milk fatty acid (FA) composition as changes in cow diets can alter FA profiles (Fleming et al., 2018) and, consequently, milk processing and storage characteristics of milk products (Bobe et al., 2003). Milk FA profile can also have implications on human health. For example, saturated FA (SFA) in dairy products have been linked with obesity, diabetes, cardiovascular disease and cancer in humans (Lavie et al., 2009; Shingfield et al., 2013). In contrast, polyunsaturated FA (PUFA), such as conjugated linoleic acid (CLA, cis-9, trans-11 CLA), a-linolenic acid (ALA, C18:3 c9,12,15) and eicosopentaenoic (C20:5 c5,8,11,14,17), have been linked with improved immunocompetence, prevention of carcinogenesis, and other positive health effects in humans (Connor, 2000). Hence, ruminant products low in SFA content but high in CLA and PUFA are regarded to be beneficial for improving long-term human health (Dewhurst et al., 2006). Polyunsaturated FA in ruminant products depend on the supply of their precursors in the diet and the extent of biohydrogenation in the rumen (Chilliard et al., 2000a). Therefore, the objective of the present experiment was to investigate the effects of feeding chicory and plantain relative to the traditional RGWC on dry-matter intake, milk yield and major milk FA in late lactating dairy cows.

4.2 Materials and methods

4.2.1 Animals and management

The experiment was conducted between 28 February and 20 March 2018 at the Lincoln University

Research Dairy Farm (43^{o}38`S, 172$^o$28`E; 17 m above sea level), with the approval of the Lincoln University Animal Ethics Committee (AEC # 2018-06). Following a 14-day baseline measurement period (14 February – 27 February 2018; when all experimental cows grazed the same diets), 54 mid-lactating Friesian · Jersey dairy cows were stratified into replicated (n = 3) groups of six cows, according to (means ± s.e.m.) milk yield (19.0 ± 1.17 kg/day), milk-solid (MS) production (milk fat + protein; 1.72 ± 0.01 kg MS), days in milk (178 ± 8.9 days) and liveweight (484 ± 20.0 kg), and randomly assigned to replicated paddocks of chicory, plantain or ryegrass–white clover (RGWC). All cows were orally administered Rumensin anti-bloat capsules (Elanco Animal Health, Auckland, New Zealand) and adapted to their forage treatments (Days 1–7), acclimatised at full forage allocation (Days 8–13), before the measurement period (Days 14–21).

Target allowance was 32 kg/cow.day of dry matter (DM) above ground level to maintain baseline milk production. Area allocated to each group was based on herbage mass, which was determined every 2 days by harvesting, to ground level, herbage within a 0.25-m^2 quadrat, and weighing the washed, dried (60^{o}C for 48 h) material. All cows had free access to fresh water at all times.

4.2.2 Herbage measurements

Herbage samples for chemical analysis were randomly collected to ground level, pre- and post-grazing at 0630 hours on Days 18 and 21. Samples were homogenised and subsampled for botanical and chemical analyses. Moisture content was determined by oven drying at 60^{o}C for 48 h. The remaining subsample was freeze-dried and ground and scanned using near-infrared spectrophotometry (NIRS, FOSS NIR Systems 5000, MD, USA) to predict organic matter, neutral and acid detergent fibre, crude protein (CP) and digestible organic matter in the DM [herbs were included in the calibration set, see Bryant et al. (2012) for details].

Apparent DM intake was determined by the difference between pre- and post-grazed herbage mass, which was determined by cutting all herbage within a 0.25-m^2 quadrat to ground level. Apparent N intake was determined using the CP (16% N) composition of the pre- and post-grazed mass.

4.2.3 Animal measurements

For continuous recording of grazing and ruminating time, three focal animals in each of the nine groups (total = 27) were fitted with Cowmanager SensOor ear-tags (Agis Ltd, Harmelen, The Netherlands). Milk yield was measured twice daily (0700 hours and 1430 hours) with an automated system (DeLaval Herd Management System, Tumba, Sweden). Milk subsamples were collected from individual cows during consecutive morning and afternoon milkings on Days 18 and 21. Milk samples were analysed for fat, protein and lactose contents by using Milkoscan^{TM} (Foss Electric, Tumba, Denmark). Milk urea N was determined on skimmed milk by an automated Modular P analyser [Roche/Hitachi, Basel; (Talke and Schubert, 1965)].

For FA analysis, bulk milk subsamples for each treatment were collected on Days 0, 3, 8, 11, 14, 18 and 21 and individual cows were sampled on Days 18 and 21. Fatty acid methyl esters of both homogenised whole milk (bulk and individualcowsamples) and herbage samples were prepared by transmethylation and analysed by gas chromatography (with AOC-20i auto-sampler, Shimadzu GC-2010, Japan, according to AOAC (2012) Method 2012.13) using a Varian CP742 silica capillary column (0.25 × 100 m × 0.2 mm).

4.2.4 Statistical analyses

For all analyses, data were averaged across days and animals within a replicate (n = 9). Forages, grazing behaviour and milk-production variables were compared using a one-way ANOVA of GENSTAT. In the statistical model, forage treatments were the fixed effects and replicates were random effects. Least-square means were generated and, on statistical significance ($\alpha = 0.05$) of the ANOVA, treatment mean separation was achieved using Tukey's HSD.

4.3 Results

4.3.1 Herbage characteristics

Botanical representations of chicory, plantain and RGWC in their respective pasture biomasses were 95%, 51% and 69% of the DM. In chicory, the remaining 5% biomass was largely dead material and some clover and weeds. In plantain and RGWC, the proportion of white clover was 34% and 9%, the proportion of weed was 9% and 12%, and that of dead material was 6% and 10% of DM respectively. Differences in pre-graze herbage mass resulted in respective area allocations of 140, 120 and 100 ± 4.8 m2/cow.day for chicory, plantain, and RGWC to achieve target allocation. On the basis of pre- and post-herbage masses, pasture utilisations of 53%, 55% and 46% of the DM were achieved for chicory, plantain and RGWC respectively. Except for CP and crude fat, herbages were variable in all chemical components (Table 4.1). Percentage of non-fibre carbohydrates was greater ($P < 0.05$) in chicory than in plantain or RGWC. Plantain had an intermediate percentage of non-fibre carbohydrates, which was greater than that of RGWC ($P < 0.05$).

Table 4.1. Herbage mass (kg DM/ha) and pre-grazing chemical and mineral (% of DM) composition of chicory, plantain and ryegrass/white clover (RGWC) sampled to ground level.

Item	Chicory	Plantain	RGWC	SEM	P value
Pre-graze mass	2302^{c}	2720^{b}	3240^{b}	53.7	<.001
Post graze mass	1094^{b}	1186^{b}	1779^{a}	63.3	<.001
Dry matter (% of FW)	9.51^{c}	11.6^{b}	17.0^{a}	0.98	<.001
Crude protein	19.7	19.4	19.8	0.81	0.92
Organic matter	86.7^{c}	87.9^{b}	91.0^{a}	0.28	<.001
Acid detergent fibre	16.6^{a}	18.1^{b}	23.8^{a}	0.39	<.001
Neutral detergent fibre	19.4^{c}	26.5^{b}	41.9^{a}	0.53	<.001
Crude fat	4.01	4.32	4.04	0.18	0.41
Water soluble carbohydrate	15.2^{a}	10.1^{b}	15.9^{a}	0.52	<.001
Non-fibre carbohydrate[1]	43.6^{a}	37.7^{b}	25.3^{c}	0.86	<.001
DOMD (% of DM)	78.4^{a}	76.5^{b}	75.7^{b}	0.41	0.01
DMD (% of DM)	85.9^{a}	81.4^{b}	78.0^{c}	0.42	<.001
OMD (% of DM)	91.4^{a}	86.0^{b}	82.6^{c}	0.58	<.001
ME (MJ kg^{-1} DM)	12.6^{a}	12.2^{b}	12.1^{b}	0.07	0.01

$^{a-c}$ Means within a row with different superscripts differ ($P < 0.05$). Where SEM is standard error of the mean, DM is dry matter, DOMD is digestible organic matter in the dry matter and DMD is dry matter digestibility, ME is metabolisable energy (0.016 × DOMD),

[1] NFC =1000- (g NDF + g CP + g CF + g Ash),

The sum of SFA was similar among the three forages (Table 4.2). While chicory contained significantly ($P < 0.05$) more PUFA than did RGWC, the sum of omega-3 FA (predominant PUFA in herbage) were greater in RGWC, than in chicory or plantain ($P = 0.003$). This was driven by a greater ALA concentration in RGWC ($P = 0.003$) than in both herbs. However, linoleic acid (LA, C18:2 c9,12) was 1.2 and 1.7 times greater (P < 001) in chicory than in plantain and RGWC respectively.

Table 4.2. Herbage fatty acid composition (g/100g of FA) chicory, plantain and ryegrass/white clover (RGWC) sampled to ground level pre-grazing.

Item	Chicory	Plantain	RGWC	SEM	P value
C14:0 Myristic	0.20^{b}	0.39^{a}	0.32^{a}	0.02	<.001
C16:0 Palmitic	13.3	12.6	12.8	0.19	0.077
C16:1 c9 Palmitoleic	0.10	0.13	0.10	0.01	0.051
C17:0 Margaric	0.06^{b}	0.08^{a}	0.09^{a}	0.01	0.003
C18:0 Stearic	0.58^{b}	0.96^{a}	1.01^{a}	0.02	<.001
C18:1 c9 Oleic	0.79^{b}	0.77^{b}	1.19^{a}	0.05	0.001
C18:2 c9,12 Linoleic	18.2^{a}	15.4^{b}	11.0^{c}	0.23	<.001
C18:3 c6,9,12 γ-Linolenic	0.06^{a}	0.02^{b}	0.01^{b}	0.01	0.004
C18:3 c9,12,15 α-Linolenic	60.4^{b}	62.2^{b}	$65.^{4a}$	0.59	0.003
C20:0 Arachidic	0.15^{b}	0.27^{a}	0.26^{a}	0.01	<.001
C20:1 c11 Eicosenoic	0.06^{a}	0.04^{b}	0.05^{ab}	0.01	0.017
C20:3 c11,14,17 Eicosatrienoic	0.07^{b}	0.09^{a}	0.06^{c}	0.01	<.001
C22:0 Behenic	0.28^{a}	0.37^{a}	0.35^{a}	0.01	0.006
C23:0 Tricosanoic	0.11^{b}	0.17^{a}	0.13^{b}	0.01	0.003
C22:2 c13,16 Docosadienoic	0.09^{ab}	0.08^{b}	0.10^{a}	0.01	0.048
C24:0 Lignoceric	0.48^{a}	0.34^{b}	0.27^{b}	0.02	0.002
Σ Saturated fatty acids (SFA)	15.5	15.5	15.8	0.27	0.623
Σ Monounsaturated fatty acids (MUFA)	2.7^{ab}	2.5^{b}	2.94^{a}	0.08	0.025
Σ Polyunsaturated fatty acids (PUFA)	78.8^{a}	78.0^{ab}	76.6^{b}	0.37	0.016
Σ Omega-3 fatty acids (n-3)	60.4^{b}	62.3^{b}	65.5^{a}	0.59	0.003
Σ Omega-6 fatty acids (n-6)	0.15	0.12	0.12	0.01	0.050
Total FA (g/kg DM)					

$^{a\text{-}c}$ Means within a row with different superscripts differ (P < 0.05). Where SEM is standard error of the mean.

4.3.2 Dry matter intake and milk yield

Cows grazing herbs spent more time grazing than did those grazingRGWC (553 vs 454 ± 19.8 min/cow.day). Cows grazing RGWC spent more time ruminating than did those on plantain or chicory (431, 340 and 192 ± 12.9 min/cow.day respectively; P < 0.05). Apparent DM intake of cows on herbs was greater than that of cows grazing RGWC (Table 4.3, P < 0.01). There were no treatment effects on apparent N intake. Cows grazing chicory or plantain had similar milk yield and MS production, which were greater (P < 0.05) than those for cows grazing RGWC. Increased MS from cows grazing chicory and plantain resulted from an increased daily production of fat (P

= 0.04) and protein (P = 0.02).

Table 4.3. Apparent intake (kg cow/day DM), milk yield and milk composition from cows fed chicory, plantain and ryegrass/white clover (RGWC).

Item	Chicory	Plantain	RGWC	SEM	P value
Apparent DMI (kg cow/day DM)	17.9^{a}	17.5^{a}	15.6^{b}	0.43	0.01
Apparent N intake (g/cow/day N)	536	498	525	17.3	0.21
Milk yield (kg/cow/day)	19.3	19.7	17.1	0.68	0.07
Milk solids (kg MS/cow/d)	1.90^{a}	1.95^{a}	1.66^{b}	0.05	0.02
Lactose %	5.02	5.08	4.98	0.03	0.20
Protein %	4.37	4.42	4.39	0.11	0.95
Fat %	5.60	5.53	5.31	0.30	0.78
Protein:Fat	0.80	0.83	0.85	0.04	0.70
Fat (g/cow/d)	1.06^{a}	1.08^{a}	0.91^{b}	0.04	0.04
Protein (g/cow/d)	0.84^{ab}	0.87^{a}	0.75^{b}	0.02	0.02
Lactose (g/cow/d)	0.97	1.01	0.86	0.04	0.11
Milk urea N (mmol/L)	8.10^{a}	7.08^{b}	9.4^{a}	0.43	0.04

$^{a\text{-}b}$ Means within a row with different superscripts differ (P < 0.05). Where SEM is standard error of the mean. Milk solids = fat (kg/cow/d) + protein (kg/cow/d).

4.3.3 Milk FA profile

Milk from cows grazing chicory and plantain had a similar percentage of PUFA, which was 68% greater than that from cows onRGWC (Table 4.4;P<0.001). Omega-3 FA concentration was greatest in milk from chicory-fed cows, and lowest in milk from RGWC-fed cows (P < 0.05). This reflects the increased proportions of ALA, which was 1.2 and 1.8 times greater (P < 0.001) in milk produced from chicory-fed cows than in that from plantain- or RGWC-fed cows, respectively. Linoleic acid followed a similar trend, as concentrations in milk were greater from cows fed chicory diet, than in milk from cows fed plantain or RGWC (P < 0.05). Concentrations of vaccenic acid [**VA** (trans-11 C18:1)] and CLA were similar in milk from cows grazing both herbs, but concentrations in milk from cows on RGWC was 1.8 times higher than from cows grazing both herbs (P < 0.05). The proportion of odd- and branched-chain FA were low in the milk (Table 4.5). The sum of odd- and branched-chain FA was similar in milk produced from cows grazing RGWC and plantain, but greater than that in milk produced from cows grazing chicory (P < 0.05). Odd-chain FA were greater in milk produced from cows grazing plantain than in milk produced from cows grazing chicory or RGWC (P = 0.001).

Changes in synthesis of key milk FA, following introduction to a new diet, occurred within a 10-day period (Figure 4.1). Relative to RGWC, the concentration of VA and CLA in milk from cows grazing the herbs decreased as the proportion of the herbs in the diet increased, reaching steady-state by Day 14 of the experiment. By contrast, LA and ALA concentrations in milk from cows grazing the herbs, increased immediately when cows commenced adaptation to herbs, reaching steady-state by Day 14.

Table 4.4. Major milk FA composition (g/100 g of FA) from cows fed chicory, plantain and ryegrass/white clover (RGWC).

Item	Chicory	Plantain	RGWC	SEM	P value
C4:0 (Butyric)	1.58	1.56	1.55	0.01	0.80
C6:0 (Caproic)	1.68	1.69	1.55	0.01	0.120
C8:0 (Caprylic)	1.25[a]	1.29[a]	1.09[b]	0.001	0.012
C10:0 (Capric)	3.37[a]	3.56[a]	2.76[b]	0.10	0.001
C12:0 (Lauric)	4.02[a]	4.37[a]	3.29[b]	0.20	0.001
C14:0 (Myristic)	11.1[ab]	11.7[a]	11.0[b]	0.20	0.043
C14:1 c9 (Myristoleic)	0.89[b]	1.03[ab]	1.07[a]	0.04	0.036
C16:0 (Palmitic)	32.7	33.8	32.5	1.10	0.690
C16:1 c9 (Palmitoleic)	1.39	1.46	1.37	0.10	0.790
C18:0 (Stearic)	8.84	7.86	9.59	0.50	0.090
C18:1 t9 (Elaidic)	0.17	0.16	0.16	0.001	0.530
C18:1 t11 (Vaccenic)	1.49[b]	1.75[b]	2.88[a]	0.10	<.001
C18:1 c9 (Oleic)	14.1[ab]	12.5[b]	15.9[a]	0.57	0.015
C18:2 c9,12 (Linoleic)	1.87[a]	1.63[b]	0.86[c]	0.05	<.001
C18:3 c6,9,12 (γ-Linolenic)	0.041[a]	0.04[a]	0.03[b]	0.002	0.007
C18:3 c9,12,15 (α-Linolenic (ALA)	2.16[a]	1.82[b]	1.03[c]	0.08	<.001
C20:0 (Arachidic)	0.10	0.10	0.11	0.01	0.067
C20:1 c9 (Gadoleic)	0.07[b]	0.08[b]	0.10[a]	0.001	0.002
C20:1 c11 (Eicosenoic)	0.04[a]	0.03[b]	0.02[b]	0.001	0.003
C20:3 c8,11,14 (Dihomo-γ-linolenic	0.054[a]	0.054[a]	0.037[b]	0.002	0.002
C20:3 c11,14,17 (Eicosatrienoic)	0.028[a]	0.025[ab]	0.019[b]	0.002	0.009
C20:4 c5,8,11,14 (Arachidonic)	0.053[a]	0.053[a]	0.036[b]	0.002	0.002
C20:5 c5,8,11,14,17 (Eicosapentaenoic)	0.11[a]	0.12[a]	0.082[b]	0.002	<.001
C22:0 (Behenic)	0.074	0.065	0.069	0.02	0.250
C22:1 c13 (Erucic)	0.079	0.069	0.072	0.001	0.210
C22:2 c13,16 (Docosadienoic)	0.0095	0.008	0.008	0.001	0.620
C22:5 c7,10,13,16,19 (Docosapentaenoic)	0.15[a]	0.13[ab]	0.11[b]	0.007	0.025
C24:0 (Lignoceric)	0.072[a]	0.05[b]	0.047[b]	0.01	0.010
C24:1 c15 (Nervonic)	0.004[b]	0.001	0.015[a]	0.001	<.001
C26:0 (Cerotic)	0.024	0.042	0.048	0.02	0.052
Conjugated linoleic acid (c9 t11 CLA)	0.67[b]	0.77[b]	1.30[a]	0.061	<.001
Σ Saturated FA (SFA)	67.7	69.7	66.8	1.32	0.156
Σ Monounsaturated FA (MUFA)	20.8[ab]	19.2[b]	22.0[a]	0.91	0.054
Σ Polyunsaturated FA (PUFA)	5.68[a]	5.08[a]	3.19[b]	0.14	<.001
Σ omega-3 FA (n-3)	2.51[a]	2.15[b]	1.30[c]	0.11	<.001
Σ omega-6 FA (n-6)	0.38[a]	0.36[a]	0.27[b]	0.01	0.004
Σ omega-9 FA (n-9)	0.23[a]	0.20[b]	0.18[c]	0.01	0.001

[a-c] Means within a row with different superscripts differ ($P < 0.05$). Where SEM is standard error of the mean.

Table 4.5. Odd and branched chain milk FA composition (g/100 g of FA) from cows fed chicory, plantain and ryegrass/white clover (RGWC).

Fatty acid	Chicory	Plantain	RGWC	SEM	P value
C5:0 (Valeric)	0.016^{ab}	0.025^{a}	0.010^{b}	0.001	0.019
C7:0 (Enanthic)	0.022^{ab}	0.035^{a}	0.016^{b}	0.002	0.016
C9:0 (Pelargonic)	0.035^{ab}	0.054^{a}	0.026^{b}	0.001	0.011
C11:0 (Undecylic)	0.067^{b}	0.102^{a}	0.045^{b}	0.001	0.005
C13:0 (Tridecylic)	0.111^{a}	0.161^{b}	0.096^{b}	0.001	0.006
C13:0 anteiso	0.167^{ab}	0.197^{a}	0.161^{b}	0.001	0.033
C13:0 iso	0.033^{b}	0.031^{b}	0.043^{a}	0.001	0.001
C14:0 iso	0.093^{b}	0.091^{b}	0.118^{a}	0.001	0.002
C15:0 (Pentadecylic)	1.39^{b}	1.69^{a}	1.45^{ab}	0.10	0.021
C15:0 anteiso	0.573	0.594	0.639	0.01	0.225
C15:0 iso	0.161^{b}	0.164^{b}	0.237^{a}	0.01	<.001
C17:0 (Margaric)	0.476^{b}	0.539^{a}	0.529^{a}	0.001	0.012
C17:0 anteiso	0.550	0.543	0.547	0.001	0.959
C17:0 iso	0.391^{ab}	0.346^{b}	0.433^{a}	0.001	0.022
C18:0 iso	0.021^{b}	0.034^{a}	0.023^{b}	0.001	<.001
C19:0 (Nonadecylic)	0.810^{b}	0.980^{a}	0.952^{a}	0.001	0.008
C23:0 (Tricosylic)	0.056	0.054	0.047	0.010	0.143
Σ Odd and branched chain FA	5.24^{b}	5.90^{a}	5.64^{a}	0.078	0.003
Σ Branched chain FA	2.47	2.26	2.26	0.441	0.077
Σ Odd chain FA	2.99^{b}	3.64^{a}	3.17^{b}	0.068	0.001
Σ iso	0.97^{b}	0.93^{b}	1.12^{a}	0.029	0.007
Σ anteiso	1.29	1.33	1.35	0.033	0.551

$^{a\text{-}c}$ Means within a row with different superscripts differ (P < 0.05). Where SEM is standard error of the mean.

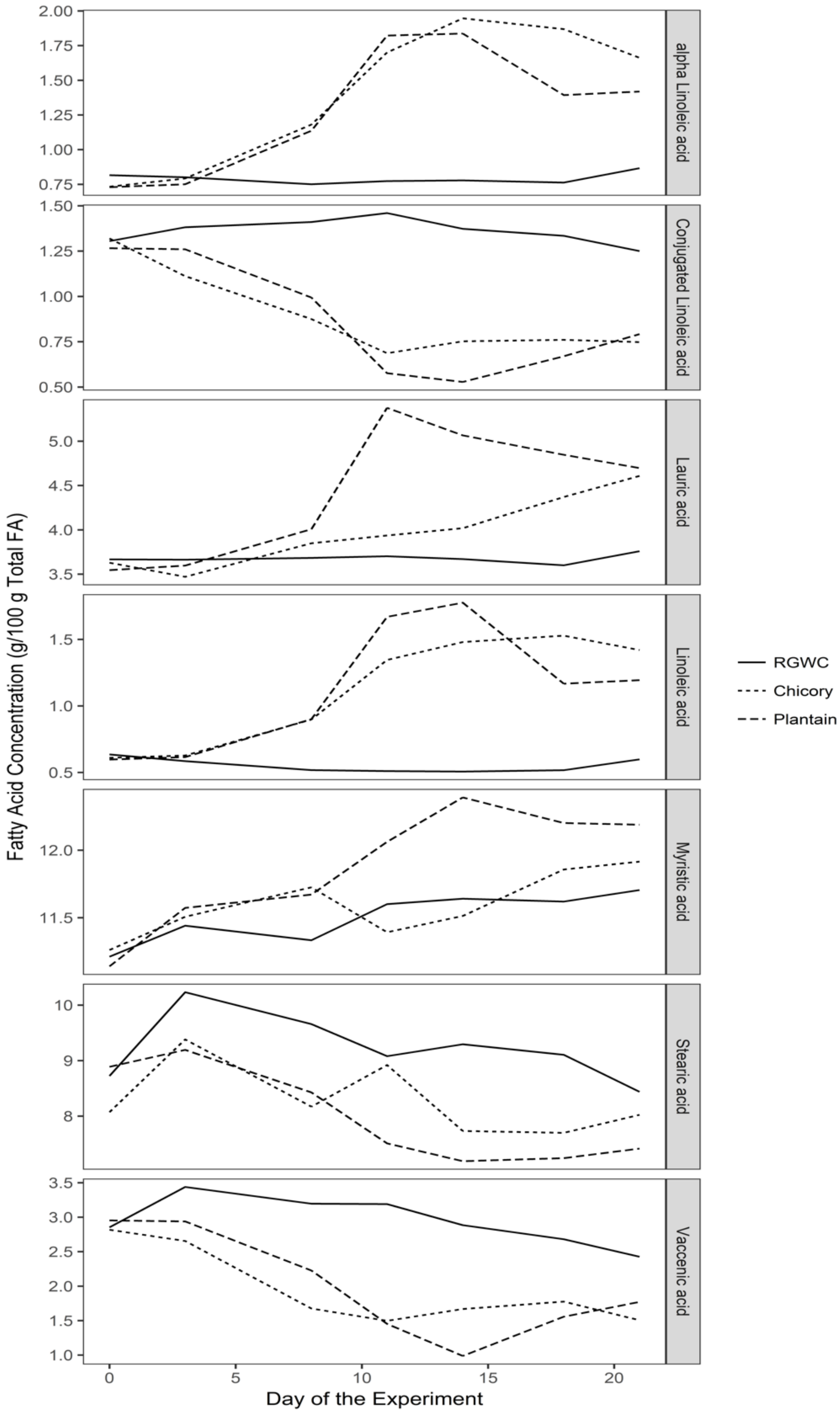

Figure 4.1. Changes over time of selected FA concentration (g/100g of total FA) in milk from cows fed chicory, plantain and ryegrass/white clover (RGWC). Areas between shaded areas in the figure represent days when cows were first adapted to their forage treatments by increasing the relative proportion of herb in the diet (day 1 to 7), acclimatised at full forage allocation (day 8 to 13), and the measurement period (day 14 to 21). Points are values for each forage type at each day of bulk milk sampling.

4.4 Discussion

Milk-solid production was greater in cows grazing chicory and plantain than in cows grazing RGWC (1.9 vs 1.7 kg MS/cow.day). However, MS production per kg DM intake was similar for all treatments (0.11 kg/kg apparently eaten), reinforcing the advantage of feeding forages with a high feeding value (Waghorn et al., 2007; Chapman et al., 2008; Minneé et al., 2012), which enabled greater utilisation of feed on offer. Moreover, the high digestibility and ratio of non-structural to structural carbohydrates observed for the herbs in the current experiment, compared with RGWC, also indicated improved energy supply, resulting in a greater milk-solid production.

Feeding forage herbs resulted in higher concentrations of milk PUFA and omega-3 FA, than did the traditional RGWC diet. The increased concentration of omega-3 FA in milk from cows grazing chicory compared with RGWC is consistent with earlier findings (Muir et al., 2014) and confirms the potential benefit of including chicory in swards. The present study is the first to compare the milk profile from cows grazing plantain and RGWC, and has shown that the greater proportion of omega-3 FA in milk from cows grazing plantain make this herb an interesting option for further investigation in farm systems.

Linoleic acid and ALA were the main PUFA in milk fat and were greater in milk from cows grazing herbs than in milk from cows grazing RGWC. The larger concentration of LA in milk from cows grazing herbs was associated with a greater LA content of herbage. Interestingly, although RGWC herbage contained more ALA than did herbs, the concentration of ALA in the milk was lower, suggesting that apparent transfer of PUFA from herbage to milk was greater on herb diets than on RGWC. Typically, transfer of LA and ALA from herbage to milk is low at less than 10% due to extensive ruminal biohydrogenation (Chilliard et al., 2001); in the present study, the apparent transfer of LA from herbage to milk increased from 7.8% for RGWC to 10.5% and 10.3% for plantain and chicory, while ALA increased from 1.6% for RGWC to 2.9% and 3.6% for plantain and chicory respectively.

Because rumen biohydrogenation of bothLAandALAforms vaccenic acid (Chilliard et al., 2000a), vaccenic acid tends to accumulate in the rumen (Lock and Bauman, 2004) and, so, its proportion in the milk depends on the supply of both ALA and LAin the diet and extent of ruminal biohydrogenation. However, the proportion of LA in the herbage was substantially greater in the herbs than in RGWC, which does not explain the greater proportion of VA in milk from cows grazing RGWC. Although the proportion of ALA was significantly greater in the RGWC herbage than in the herbs, the differences in herbages do not explain the 64.6 – 93.2% difference inVAin milk. The proportion of CLA was greater in milk from cows grazing RGWC. The precursor of CLA, i.e. LA (and even ALA as a precursor of VA), is reduced in milk from cows grazing RGWC, while products further along the hydrogenation pathway (VA, stearic acid) are in much greater supply than they are in milk from herbs.

The changes in milk fat composition in cows grazing herbs suggest that feeding herbs reduces biohydrogenation, which may be a result of greater digestible organic matter in the DM and faster ruminal passage of herbs than of RGWC. Higher ruminal-passage kinetics reduces exposure of forage lipids to lipases and biohydrogenation in the rumen (Dewhurst et al., 2003). Although we did not measure feed passage rate in the current study, the low fibre and high moisture content of the herbs (Table 4.1) and changes in grazing and ruminating behaviour leading to increased intake (Table 4.3) indicated either faster rumen degradation or more rapid rumen clearance on herb diets than on grass-based diets. Similar findings by Kusmartono et al. (1997) who reported a higher rumen fractional degradation rate and fractional outflow rate in deer fed chicory than in those fed RGWC, and by (Box et al., 2018) who reported faster in sacco ruminal passage kinetics in cows fed plantain than in those fed RGWC further corroborate this explanation.

Another explanation for the observed differences in milk fat composition may be the variation in ruminal pH. Diets rich in readily degradable carbohydrates are known to reduce pH during ruminal fermentation, restricting lipolysis and, thus, biohydrogenation (Chouinard et al., 1999). Although pH was not measured in the current study, the larger content of readily fermentable carbohydrates in the herbs than in the RGWC may have lowered the rumen pH, resulting in an increase in PUFA content of milk. Indeed, (Minneé et al., 2017) reported a rapid decline in pH after afternoon feeding and lower pH values between 2000 hours and 2300 hours from cows fed chicory and plantain than from those on the RGWC diet.

4.5 Conclusions

The present findings have demonstrated that feeding chicory or plantain can increase apparent DM intake, milk production and PUFA in milk, including omega-3 FA, compared with conventional ryegrass and white clover diets. Further investigation is recommended to ascertain the forage effects on ruminal fermentation characteristics and biohydrogenation.

Chapter 5

5. Functional traits, morphology and herbage production of vernalised and non-vernalised chicory cv. choice (*Cichorium intybus* L.) in response to defoliation frequency and height

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The content of this Chapter is exact copy of the journal paper referred to above. The original paper has been formatted for consistence within the thesis.

5.1 Introduction

The challenges of maintaining productivity while meeting regulations for improved environmental outcomes and reducing water usage in pasture-based systems in New Zealand relying on perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) highlights the need to consider the role of alternative forages in facing these issues. Recent research has shown environmental benefits from forages based on a range of plant traits including winter activity for reduced nitrate leaching (Malcolm et al., 2018); high moisture content to reduce urinary nitrogen load (Bryant et al., 2019); low fibre content to reduce methane (Gregorini et al., 2016), and increased soil water use to reduce drainage (Brown et al., 2003; Welten et al., 2019). Forage herbs such as chicory (*Cichorium intybus* L.) possess many of the attributes required to improve pastoral farm systems. Grasslands Choice is one of the two newer varieties of chicory available for commercial use on pastoral systems (Lee et al., 2015a). Grasslands Choice was bred from 'Grasslands Puna' chicory with selection emphasis on lower levels of sesquiterpene lactones, a compound that causes a taint or bitter aftertaste in the milk (Rumball et al., 2003b). Chicory (cv. Choice) fed to dairy cows has shown to increase water intake and reduce urinary nitrogen concentration (Mangwe et al., 2019) in addition to supporting greater milk production (Minneé et al., 2017; Mangwe et al., 2020b) and enhance milk fatty acids profile (Muir et al., 2015; Mangwe et al., 2020a). However, the adoption of alternative forages in different grazing systems requires confidence in the response to management decisions and needs to identify risks to production in different environments.

The development of the reproductive stem in winter vernalised chicory plants has slowed its adoption in pastoral systems (Li and Kemp, 2005; Lee et al., 2015a). Mature reproductive stems of perennial herbaceous plants decrease forage quality due to a lowered leaf-to-stem ratio within individual plants and the more structural carbohydrates in mature stems (Chen et al., 2019; Ta et al., 2020). This change in plant biomass partitioning and chemical and structural composition reduces feeding value of the plant (Clark et al., 1990b; Gregorini et al., 2013). By contrast, mechanisms and factors that trigger and influence the development and elongation of reproductive stems in vernalised chicory have been poorly studied. (Clapham et al. (2001) investigated the development dynamics of vernalised chicory cv. Puna in relation to thermal time and reported that time to bolting was 400 growing degree-days (GDD). Little is known about the development dynamics (i.e., bolting initiation and stem elongation) of the commercially available chicory cv.

Choice. If the objective is to control the growth and development of the reproductive mature stems, chicory should be grazed before bolting to maintain plants in the vegetative state (Barry, 1998b). Understanding the morphological and physiological response to defoliation before bolting, i.e., 300 GDD or after bolting, i.e., 600 GDD and the traits associated with this is therefore strongly desirable to generate efficient and specific defoliation strategies of chicory pastures that could be used to control production and feeding value of chicory on farm.

Another key limitation of chicory is limited persistence, though range in longevity has been reported in a review to be between 3 and 7 years (Li and Kemp, 2005), mainly dependent on suitability of climate, establishment and defoliation management. While there is a substantial volume of information available on the management options and associated impacts on forage yield of chicory in New Zealand and USA (Li et al., 1997c; a; Labreveux et al., 2004; Lee et al., 2015a), there is poor information on forage production, morphology, and physiology of grazed chicory. Dairy cows have the potential to exacerbate treading impact and reduce plant density due to greater treading damage (Glassey et al., 2013). Additionally, most studies were conducted in rainfed environments without irrigation. In natural rain fed environments, where summer moisture deficits are common, chicory has shown to be more sensitive to defoliation interval than defoliation height (Li et al., 1997a; Labreveux et al., 2004; Lee et al., 2015a). The partitioning of photosynthates to above ground biomass of chicory is reduced under moisture deficit (Monti et al., 2006; Cranston et al., 2016), so morphological response of chicory (to defoliation interval and/or height) may differ in an irrigated environment.

Morphological, physiological and functional traits have been useful to characterize plant species regarding their strategies to acquire, store, and invest nutrients and energy as well as to respond to variable frequency and height of defoliation (Moreno García et al., 2014; Lee et al., 2015b; Cranston et al., 2016). The shoot size-shoot density dynamics have been particularly relevant to categorize plants according to their response to disturbance (Gastal and Lemaire, 2015). The shoot size-shoot density dynamic is determined by several major morphological traits such as leaf and stem mass, which are directly and indirectly associated to the overall plant fitness, persistence and herbage production (Violle et al., 2007). The final number of leaves, leaf length, specific leaf area and overall absolute mass of leaves per plant are influenced by leaf appearance and elongation rates (Duru and Ducrocq, 2000). Leaf elongation is also important for maintaining the overall productivity of the plant and, therefore, the sward in general (Durand et al., 1999). At species level, studies have shown that leaf elongation rate differs, depending on factors such as temperature and plant phenology stage; reproductive or vegetative (Granier et al., 2002; Walter and Schurr, 2005). To our knowledge, no studies have investigated the leaf elongation rate of Choice chicory in response to varying regrowth interval and at different phenological stages.

Therefore, we conducted two experiments to examine the effects of defoliation regimes in irrigated dairy pastures on functional traits, morphology and herbage production of chicory, before and after vernalisation. The main aim of the first experiment was to compare the effects of two regrowth intervals, based on growing degree-days (GDD), and two defoliation heights on functional traits, morphology, and herbage production of vernalised and non-vernalised chicory cv. Choice. The second experiment measured the effects of the two regrowth intervals on leaf photosynthetic capacity and the development dynamics of leaves and stems.

5.2 Materials and methods

5.2.1 Site description and establishment of chicory cv. Choice

Two experiments were undertaken at the Lincoln University Research Dairy Farm in Canterbury, New Zealand (43°38′S, 172°28′E; 17 m above sea level). The soil is classified as free-draining Templeton fine sandy loam soil with soil pH of 6.3 (1: 2.1 *v/v* soil–water slurry), Olsen phosphorus 30 mg/L, potassium 8.7, calcium 8.7, magnesium 0.19, and sodium 0.2 me/100 g as determined to 75 mm depth on 29 September, 2017. The experimental areas consisted of 10 × 40 m fenced areas within four larger 1.5 ha paddocks. Site preparation before planting involved spraying existing perennial ryegrass and white clover pastures with Weedmaster 540 (540 g/L glyphosate; Nufarm Limited, Auckland, NZ) at 2 litres/ha with a surfactant, Pulse™ at 100ml/100 litres of water on the 21 September 2017. Paddocks were then chip-hoed and ploughed on 20 October 2017, with chicory (cv. Choice) sown at 5.3 kg seed/ha on 17 November 2017. No fertiliser was used during establishment. For post-emergence weed control, Preside™ (800 g/kg flumetsulam; Dow Agroscience Limited, New Plymouth, NZ) was used after the emergence of the fourth leaf with the addition of a spraying oil at 500 ml/100 litres water.

The first defoliation occurred on 16 January 2018 when plants had at least seven fully developed leaves (Powell et al., 2007). Defoliation was carried out by grazing with dairy cows (Plate 5.1) for a maximum of three hours to minimize damage. The second defoliation occurred on 15 February 2018, by grazing with dairy cows for several hours followed by mowing to a uniform height of 4 cm.

Plate 5.1. Dairy cows grazing vegetative chicory herbage.

5.2.2 Management of chicory (Experiment 1)

The experiment was conducted between 15 February 2018 and 5 February, 2019 in pre-established plots of chicory cv. Choice. Treatments included two regrowth intervals based on the accumulated GDD, i.e., every 300 and 600 GDD, and two defoliation intensities at 4 and 8 cm stubble height. Treatments were applied in a completely randomized block design with four replications. In each of the four paddocks (blocks), defoliation regimes were randomly allocated to four 10 m × 10 m plots. Regrowth intervals in GDD were calculated above a 4 °C base temperature as:

$$\text{GDD} = (\text{daily maximum temperature} + \text{daily minimum temperature})/2) - 4))$$

The temperature was set to equal 4 °C when the mean daily temperature was below 4 °C (McMaster and Wilhelm, 1997). Climatic data, including air temperature and rainfall were recorded at the NIWA weather station in Lincoln (Broadfield met station #17603), 2 km away from the experimental site. Monthly climate values during the experiment and a 10-year mean preceding the experiment are presented in Table 5.1. All plots were irrigated as necessary based on soil moisture data using the centre pivot system used by the farm. Monthly accumulated irrigation water applied during the experiment is presented in Table 5.1. Three applications of nitrogen fertiliser were applied as urea immediately after grazing all experimental plots in February, October, and December 2018 at doses of 30, 50, and 30 kg N/ha, respectively.

Table 5.1**.** Monthly accumulated precipitation, irrigation water applied and mean air temperature in Lincoln, Canterbury, New Zealand during the experiment (Exp.) between February 2018 and March 2019. The 10-year means (Avg.) were calculated based on data from January 2008 to December 2017.

	Total Precipitation (mm)		**Irrigation (mm)**	**Avg. Max temp (°C)**		**Avg. Min Temp (°C)**	
Month	**During Exp.**	**10-yrs Mean**	**During Exp.**	**During Exp.**	**10-yrs Mean**	**During Exp.**	**10-yrs Mean**
Feb-18	123	28.3	17.8	22.5	22.0	12.1	11.8
Mar-18	31.6	57.1	0.4	20.7	20.1	11.4	10.0
Apr-18	91.0	65.6	2.3	17.6	17.7	6.7	7.8
May-18	47.6	77.0	0	14.3	14.8	5.4	5.1
Jun-18	58.8	72.8	0	10.6	11.9	3.8	2.5
Jul-18	26.2	43.9	0	13.0	11.4	2.3	1.5
Aug-18	15.8	57.6	0	13.0	12.7	3.6	3.6
Sep-18	39.4	34.6	0	14.3	14.6	4.7	4.8
Oct-18	55.6	50.8	10.5	16.9	16.6	6.1	6.5
Nov-18	107.4	33.0	2.1	18.1	18.8	8.6	8.3
Dec-18	57.0	44.2	2.1	19.4	20.9	12.3	11.0
Jan-19	36.2	44.5	24.1	23.9	22.1	13.1	11.8
Feb-19	29.2	28.3	44.8	24.3	22.0	11.6	11.8
Mar-19	23.8	57.1	14.8	21.8	20.1	11.9	10.0
Total / Ave	**742.6**	**694.8**	**118.9**	**17.9**	**17.6**	**8.1**	**7.6**

Grazing events were restricted to a maximum of 5 hours with 2 to 6 dairy cows (484 ± 16.9 kg live weight kg) to achieve the target defoliation height of each treatment. Cows were moved out of respective plots once the corresponding stubble height was reached based on a visual estimation (Plate 5.2). Cows were left for longer periods on the 4 cm treatments to ensure plots had significantly less leaf material than the 8 cm treatment plots (Cranston et al., 2015). Plots were not grazed during wet conditions or during winter to limit soil and plant damage and to improve plant survival (Li and Kemp, 2005). During the experiment, the 300 GDD plots were grazed eight times with an average GDD of 326 ± 31 and 600 GDD plots were grazed five times with an average of 653 ± 32 GDD (Table 5.2). The first grazing for 300 GDD in March 2018 was delayed by two weeks (180 GDD) due to resources being committed to a separate experiment at the experimental site (Table 5.2). The first defoliation post vernalisation was due to occur in early September 2018 when accumulated thermal time was reached but was delayed until early October due to insufficient herbage. Defoliation in October was a combination of grazing followed by mowing to respective treatment heights (4 and 8 cm).

Plate 5.2.Chicory herbage grazed to 8 cm (A) and 4 cm (B) by dairy cows.

Table 5.2. Defoliation dates and accumulated growing degree-days (GDD) between grazing events applied from February 2018 and January 2019.

Year	Harvest Period	Defoliation Intervals			
		300 GDD		600 GDD	
		Date	Accumulated GDD	Date	Accumulated GDD
2018	1	15-Feb		15-Feb	
	2	26-Mar	480		
	3	28-Apr	298 [1]	6-Apr	609 [1]
	4	5-Oct	740	5-Oct	907
	5	13-Nov	320 [1]		
	6	13-Dec	298 [1]	13-Dec	636 [1]
2019	7	8-Jan	313 [1]		
	8	5-Feb	402 [1]	5-Feb	715 [1]

[1] Values used to calculate the average of accumulated GDD between grazing events for each treatment: 326 for 300 GDD and 653 for 600 GDD plots.

5.2.3 Morphological and physiological measurements (Experiment 1)

For comparison of treatment effects on morphological and physiological traits, six random plants per plot replicate were measured on three occasions between February 2018 and February 2019; once before vernalisation in autumn 2018 (representing regrowth in February and April) and twice after vernalisation in spring 2018 and summer 2019 (spring regrowth between October and November, and summer regrowth December to February). Each plant was dug from the ground (Plate 5.3) and separated into roots, shoots (primary and secondary), alive leaves and dead material (leaf plus stem dead material). Primary shoots were defined as those growing from the crown (Plate 5.4) while secondary shoots were defined as the lateral shoots on the primary shoots (Li et al., 1997c). The roots were washed and the taproot diameter (mm) of each plant was measured at the top widest part of the taproot. Due to difficulties in digging the whole taproot intact, only the top 15 cm of the taproot was washed and oven dried at 60 °C for 48 h to determine dry weight and water soluble carbohydrates (WSC) content (Pollock and Jones, 1979). Aboveground herbage was oven dried separately at 60 °C and dry weight was recorded.

5.2.4 Herbage production measurements (Experiment 1)

Pre- and post-herbage mass was determined by harvesting to ground level all herbage within three 0.25-m^2 quadrats before and after each defoliation event (3 × 4 reps = 12 quadrats per treatment). Accumulated herbage mass was calculated as the difference between pre-graze mass of current grazing event and post-graze mass of previous grazing event. Plant density in each plot was determined once, before vernalisation (autumn 2018) and twice after vernalisation (spring 2018 and summer 2019) by counting the number of plants within a 0.25-m^2 quadrat immediately after a grazing event.

Plate 5.3. Vegetative chicory plant dug from the ground.

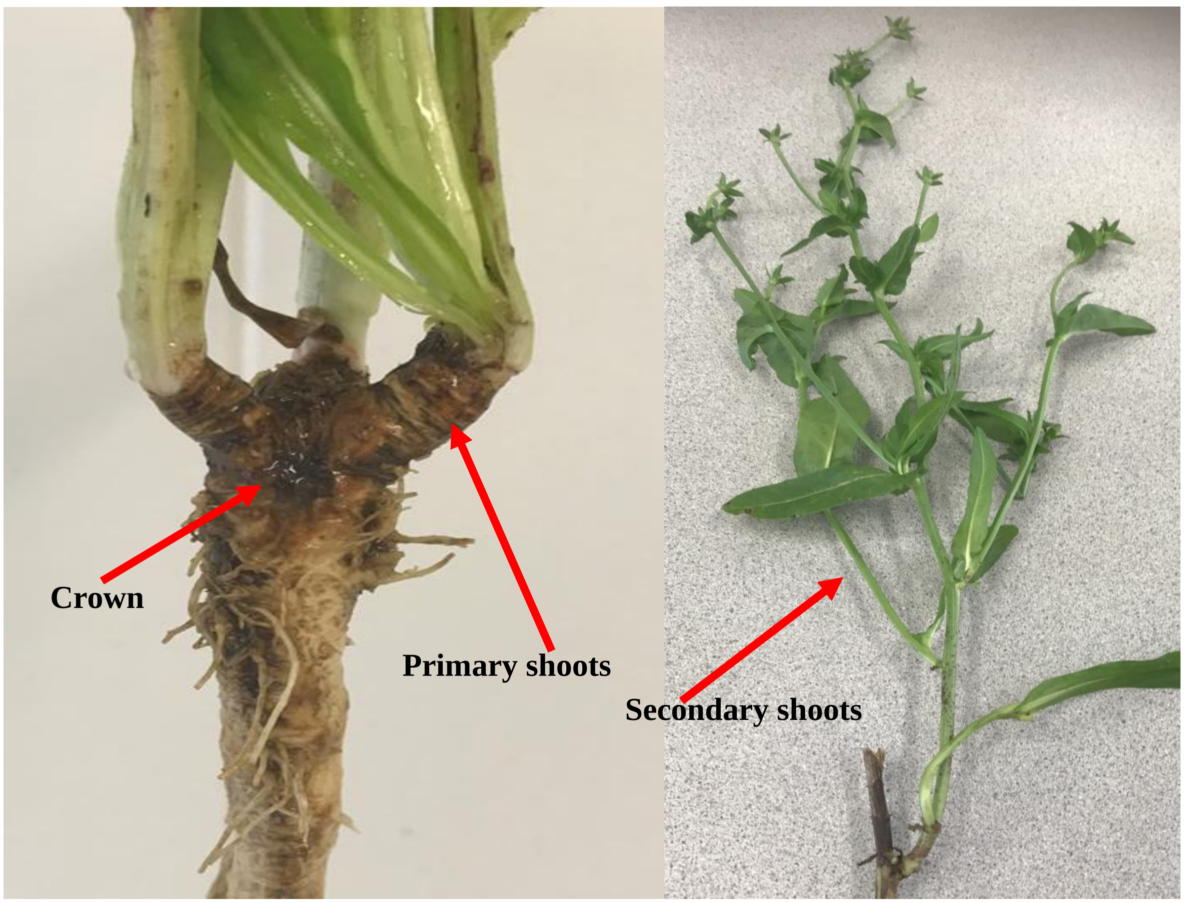

Plate 5.4. Crown, primary and secondary shoots of chicory plants after winter vernalisation.

5.2.5 Plant function of vernalised chicory plants (Experiment 2)

Plant measurements (recorded every 2 to 3 days) commenced immediately after the final defoliation in Experiment 1 (5 February 2019) and ceased after 475 GDD (17 March 2019), when branching of selected main stems was too prolific for accurate record of measurements. For this study, we used plots from the 4 cm stubble height treatment with the corresponding regrowth intervals of 300 GDD and 600 GDD. Six plants per plot (n = 24 plants per treatment) were randomly selected and one primary shoot randomly selected from each plant was marked with coloured wire for monitoring of plant functional traits. To ensure consistency among treatments, any selected plants exceeding 4 cm height were cut to the target treatment height using scissors.

The number of visible leaf tips, length of each leaf (from the base up to the tip of the leaf), length of visible stem, and onset of senescence (decrease in green length) were recorded. Leaves were counted acropetally, with the first leaf identified as leaf 1. These measurements enabled us to estimate the number of leaves on the primary shoot over time, elongation rate of each leaf, time to bolting (stem appearance), stem elongation rate and time to senescence of old leaves. Thermal times (e.g., degree-days to first leaf appearance after defoliation, initiation of stem elongation, beginning of senescence) were determined when 50% or more of observed plants reached the aforementioned phenological stages. Leaf elongation rate (mm/GDD) was calculated as the ratio between the increase of leaf length and the accumulated GDD on two consecutive samplings.

At the end of the regrowth period, leaf chlorophyll content was measured from three reproductive plants of the 600 GDD plots and three vegetative plants of the 300 GDD plots using the Soil Plant Analysis Development (SPAD, Minolta Camera Co., Osaka, Japan) chlorophyll meter. From each selected shoot, three mature leaves were measured; we took three readings per leaf (at a third, a half and at two thirds of the distance from the base) and averaged to one value per leaf (Yuan et al., 2016). At the end of the experiment, plants holding selected shoots were dug out. Taproots were washed and their diameter measured (see Experiment 1). The number of live leaves and shoots per plant were recorded. Specific leaf area (cm^2/g) was calculated as the ratio of plant leaf area (LI-COR leaf area meter LI-3100 Li-Cor, (Lincoln, NE, USA)), and the dry weight of all the leaves. All plant components were dried at 60 °C separately and weighed to determine their absolute dry mass.

5.2.6 Data analysis

All data were analysed using R Core Team. In Experiment 1, we used mixed effect models from 'lme4' package version 1.1–21 of R (Bates et al., 2015) where plot was used as the experimental unit, regrowth interval (300 GDD and 600 GDD), defoliation height (4 cm and 8 cm) and age (before and after vernalisation) were included as fixed effects and block and sampling day as nested random effects. The 'emmeans' package version 1.4.5 of R, using the Tukey method for means separation at significance of $P < 0.05$.

In Experiment 2, developmental parameters (leaf length, leaf and stem elongation rates) were modelled as functions of cumulative GDD using polynomial mixed model regressions with the 'lmer' function of the 'lme4' package version 1.1–21 of R. Block was included as a random effect in all models. Leaf length data were also modelled as a function of leaf number (leaf position) to determine length of longest leaf. Based on the mixed model regression equations, predicted values were generated and plotted using the 'ggeffects' package version 0.14.2 of R (Lüdecke, 2018) Additionally, the derivative of the model was calculated in order to determine where the GDD, a local maximums, occurred in the respective regression equations. These values for GDD were then applied to the original regression to determine what the maximum values were. Modelled results are presented up to leaf number 7 for all treatments because leaf number eight and above in each shoot for 600 GDD plants had three or less observations during the experiment.

A principal component analysis (PCA) was used to summarize the variation of functional, morphological, and physiological traits of vernalised chicory plants under the two defoliation intervals. Dimensionality reduction analyses were conducted using the 'factoextra' package, version 1.0.7 of R (Kassambara and Mundt, 2020), with defoliation intervals as explanatory variables and plant traits as response variables.

5.3 Results

Overall, regrowth interval had the greatest effect on morphological traits and on herbage production followed by age (i.e., before and after vernalisation); defoliation height had no major effects on the aforementioned parameters (Table 5.3).

Table 5.3. Effects of regrowth interval, defoliation height and age, and interaction effects on morphological traits, plant density, and herbage production of chicory cv. Choice.

	Interval (I)[1]	**Height (H)**[1]	**Age (A)**[1]	**I × H**	**I × A**	**H × A**	**I × H × A**
			At individual Plant Level				
Number of leaves	0.006	0.777	< 0.0001	0.319	0.048	0.728	0.351
Number of primary shoots	< 0.001	0.996	< 0.001	0.706	< 0.001	0.281	0.304
Number of secondary shoots	< 0.001	0.894	< 0.001	0.240	0.001	0.796	0.240
Total shoots per plant	< 0.001	0.748	< 0.0001	0.193	< 0.001	0.423	0.308
Absolute leaf mass (g DM[2])	< 0.001	0.275	0.294	0.965	< 0.001	0.249	0.233
Absolute stem mass (g DM)	< 0.001	0.091	< 0.001	0.571	< 0.001	0.091	0.571
Total plant weight (g DM)	< 0.001	0.465	< 0.001	0.652	0.073	0.484	0.841
Root diameter (mm)	< 0.001	0.203	< 0.001	0.032	0.764	0.911	0.858
Roots WSC[3] (g/kg DM)	< 0.001	0.149	< 0.001	0.356	0.211	0.86	0.77
			At Pasture Level				
Pre graze mass (kg/ha DM)	< 0.001	0.522	0.678	0.600	0.566	0.580	0.536
Post graze mass (kg/ha DM)	0.961	0.014	< 0.001	0.798	0.033	0.434	0.306
Accumulated herbage mass	< 0.001	0.646	< 0.001	0.467	0.002	0.771	0.724
Plant density (plants/m^2)	0.778	0.496	< 0.001	0.695	0.643	0.343	0.253

[1] Interval = 300 and 600 growing degree-days; Height = 4 and 8 cm; Age = before vernalisation (February–May 2018) and after vernalisation (October 2018–February 2019); [2] Dry matter; [3]Water soluble carbohydrates.

5.3.1 Morphology of individual chicory plants

There was a regrowth interval by age interaction ($P < 0.05$) on the number of shoots and leaves per plant (Table 5.3) of chicory plants. Before vernalisation, chicory plants in all treatments had 1.4 ± 0.31 shoots per plant and remained in a rosette growth form (Table 5.4). There was no effect of treatment on leaves per plant (14.6 ± 2.11 leaves/plant) which exhibited large variation (range between 10 and 19 leaves/plant). After vernalisation, the number of shoots per plant increased (Table 4; $P < 0.0001$). For example, primary shoots were two-fold greater in 300 GDD plants than 600 GDD plants ($P < 0.0001$), while secondary shoots were approximately eight times greater for 600 GDD plants than 300 GDD plants ($P < 0.0001$). As a result, after vernalisation the total number of shoots per plant was 1.9 greater in 600 GDD plants than 300 GDD plants. Likewise, the number of leaves per plant increased after vernalisation, and were 19% greater for 600 GDD plants than 300 GDD plants, regardless of defoliation height.

There was an interaction ($P < 0.001$) between regrowth interval and age on absolute leaf and stem mass. Before vernalisation, absolute leaf mass was 57% greater for 600 GDD plants than 300 GDD plants ($P < 0.01$), regardless of defoliation height. In contrast, after vernalisation, absolute leaf mass was similar for all treatments. Plants exposed to a longer regrowth interval (600 GDD) had 2.5 times greater absolute stem mass than 300 GDD plants after vernalisation ($P < 0.0001$). As a result, total plant weight (leaf plus stem mass) was 1.4 times greater for 600 GDD plants than 300 GDD plants (Table 5.4).

There were main effects of regrowth interval and age ($P < 0.0001$) on root WSC concentration (Table 5.3). The concentration of WSC in chicory roots was 10% and 33% greater for 600 GDD plants than 300 GDD plants before and after vernalisation, respectively, regardless of defoliation height. Chicory root WSC concentration declined by nearly 46% and 35% after vernalisation for 300 GDD and 600 GDD plants, respectively (Table 5.4). There were main effects of regrowth interval and age on root diameter ($P < 0.0001$), and a defoliation height by age effect on root diameter ($P = 0.032$). Before vernalisation, the root diameter of 600 GDD chicory plants grazed to 4 cm were not different from 300 GDD treatment plants. After vernalisation, root diameter of all 600 GDD plants was greater than 300 GDD plants, regardless of defoliation height.

5.3.2 Herbage production and plant density of chicory sward

Herbage mass and plant density for all treatments before and after vernalisation are shown in Table 5.5. Pre-graze mass was consistently greater ($P < 0.05$) for 600 GDD plants than 300 GDD plants throughout the growing seasons. On average, post-graze mass was ~20.4% and 17.8% greater for 8 cm than 4 cm stubble height before and after vernalisation, respectively, regardless of regrowth interval. Post-graze mass was ~63% greater ($P < 0.0001$) after vernalisation than before vernalisation.

Table 5.4. Morphology of individual plants of chicory cv. Choice under contrasting defoliation regimes before and after vernalisation.

Interval	300 GDD[1]		600 GDD[1]		SEM[2]
Height	4 cm	8 cm	4 cm	8 cm	
Before Vernalisation (February–May 2018)					
Number of leaves (leaves/plant)	15.2	13.8	13.9	15.4	2.11
Number of primary shoots (shoots/plant)	1.58	1.25	1.33	1.25	0.31
Number of secondary shoots (shoots/plant)	0	0	0	0	
Total shoots per plant (shoots/plant)	1.58	1.25	1.33	1.25	0.7
Absolute leaf mass (g DM)	2.44 ^{b}	2.24 ^{b}	3.57 ^{a}	3.78 ^{a}	0.26
Absolute stem mass (g DM)	0	0	0	0	
Total plant weight (g DM)	2.44 ^{b}	2.24 ^{b}	3.57 ^{a}	3.78 ^{a}	0.51
Root diameter (mm)	13.4 ^{b}	14.7 ^{b}	16.5 ^{b}	18.8 ^{a}	0.83
Roots water soluble carbohydrates (g/kg DM)	756 ^{b}	766 ^{b}	803 ^{a}	870 ^{a}	38.1
After Vernalisation (October 2018–February 2019)					
Number of leaves (leaves/plant)	31.6 ^{b}	29.9 ^{b}	38.3 ^{a}	35.0 ^{a}	1.49
Number of primary shoots (shoots/plant)	3.99 ^{a}	4.46 ^{a}	2.19 ^{b}	2.12 ^{b}	0.22
Number of secondary shoots (shoots/plant)	1.38 ^{b}	0.38 ^{b}	6.97 ^{a}	8.53 ^{a}	0.62
Total shoots per plant (shoots/plant)	5.37 ^{b}	4.84 ^{b}	9.16 ^{a}	10.7 ^{a}	0.51
Absolute leaf mass (g DM)	3.53	3.19	3.01	2.98	0.18
Absolute stem mass (g DM)	1.23 ^{b}	1.77 ^{b}	3.22 ^{a}	4.30 ^{a}	0.27
Total plant weight (g DM)	4.76 ^{b}	4.96 ^{b}	6.23 ^{a}	7.28 ^{a}	0.39
Root diameter (mm)	18.1 ^{c}	19.0 ^{c}	22.8 ^{b}	26.8 ^{a}	1.30
Roots water soluble carbohydrates (g/kg DM)	402 ^{b}	417 ^{b}	522 ^{a}	568 ^{a}	38.1

$^{a–c}$ Means within a row with different superscript letters differ significantly ($P < 0.05$); [1] GDD = Growing degree-days; [2] SEM = Standard error of the mean.

There was a regrowth interval by age interaction for accumulated herbage mass ($P = 0.002$). Before vernalisation, accumulated herbage mass was not different between treatments (4357 ± 213 kg/ha of dry matter (DM) between February and May 2018), while after vernalisation between October 2018 and February 2019, 600 GDD plants accumulated 12.8% greater herbage mass than 300 GDD plants, regardless of defoliation height (Table 5.5).

Plant density (plants/m^2) declined ($P < 0.0001$) by nearly 45% after vernalisation and was not different ($P \geq 0.496$) between defoliation regimes.

Table 5.5. Plant density and herbage production of chicory stands under contrasting defoliation regimes before and after vernalisation.

Interval	300 GDD[1]		600 GDD[1]		SEM[3]
Height	4 cm	8 cm	4 cm	8 cm	
Before vernalisation (February–May 2018)					
Pre graze mass (kg/ha DM)	3510^b	3293^b	4648^a	5032^a	415
Post graze mass (kg/ha DM)	627	774	586	687	111
Accumulated herbage mass (kg/ha DM)	4192	4242	4637	4358	213
Plant density (plants/m^2)	127	124	130.5	122	8.25
After vernalisation (October 2018–February 2019)					
Pre graze mass (kg/ha DM)	3510^b	3659^b	5248^a	5407^a	293
Post graze mass (kg/ha DM)	944^c	1077^b	973^c	1182^a	78.8
Accumulated herbage mass (kg/ha DM)	10284^b	10316^b	11659^a	11576^a	320
Plant density (plants/m^2)[2]	73	71	65	68	6.36

$^{a–c}$ Means within a row with different superscript letters differ significantly ($P < 0.05$); [1] GDD = Growing degree-days; [2]Values derived from summer measurements; [3] SEM = Standard error of the mean.

5.3.3 Functional traits after vernalisation

The effects of previous defoliation management on end of season (late summer/early autumn) leaf length and elongation rates of vegetative (300 GDD) and reproductive (600 GDD) plants are presented in Figures 5.1–5.3. Following defoliation, leaves appeared sooner in 300 GDD plants than 600 GDD plants (39.1 vs. 55.5 GDD, respectively, $P < 0.05$). The relationship between leaf length and cumulative GDD was cubic ($P < 0.0001$) for both 300 GDD and 600 GDD plants (Figure 5.1), and the intercept of the predicted regression curve was similar for both treatments ($P = 0.28$). However, the mean leaf length was consistently greater for 300 GDD plants than 600 GDD plants (Figure 5.1; Figure 5.2; $P < 0.05$). Maximum length was achieved at third leaf for 300 GDD and at both second and third leaf for 600 GDD plants (Figure 5.2).

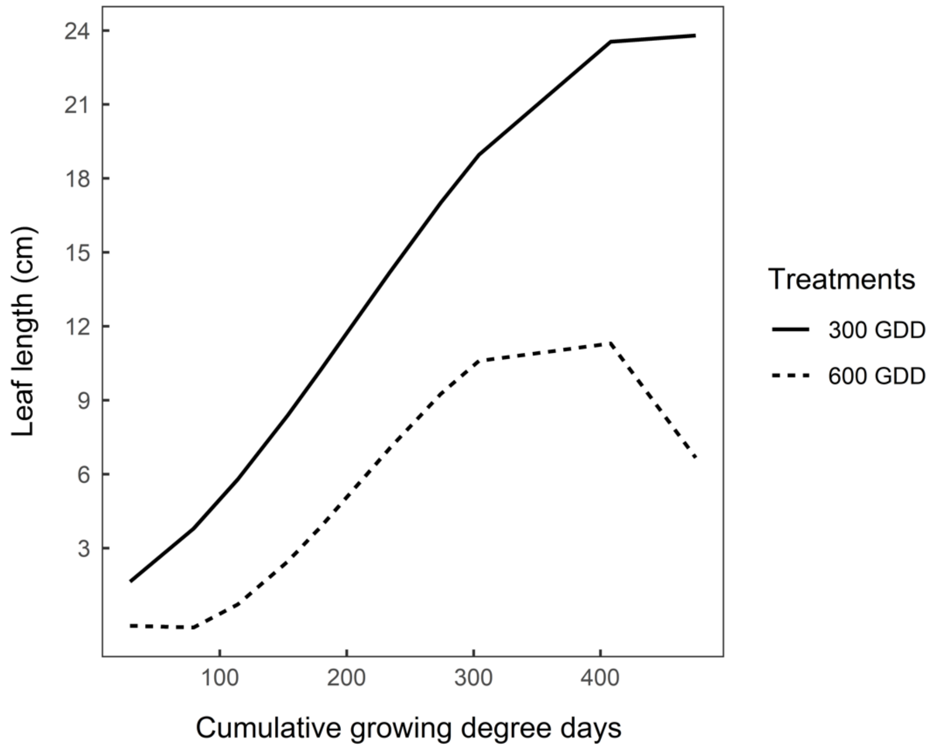

Figure 5.1. Modelled mean leaf length (cm) of the first seven leaves of 300 GDD and 600 GDD chicory plants during a regrowth between February and March 2019 (475 GDD). Lines are predicted values plotted against cumulative growing degree-days (GDD) using equations: $y = 0.88 + 0.02x + 0.00026x^2 – 0.00000042x^3$ for 300 GDD plants and $y = 0.88 – 0.049x + 0.00051x^2 – 0.00000081x^3$ for 600 GDD plants (n = 24). Root mean square prediction error = 5.4, Error due to random effects (i.e., block) = 0.61.

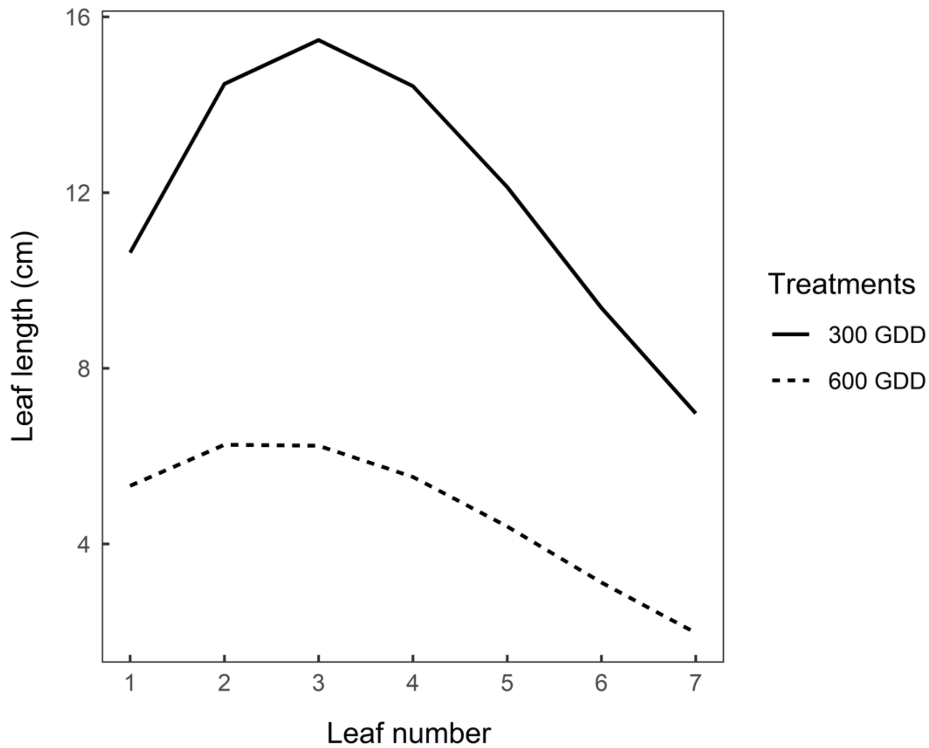

Figure 5.2. Modelled mean leaf length (cm) of each of the first seven leaves of 300 growing degree-days (GDD) and 600 GDD chicory plants during regrowth between February and March 2019 (475 GDD). Lines are predicted values plotted against leaf number (leaf position) using equations: $y = 7.85 + 9.56x - 2.2x^2 + 0.13x^3$ for 300 GDD plants and $y = 4.4 + 2.87x - 0.75x^2 + 0.05x^3$ for 600 GDD plants (n = 24). Root mean square prediction error = 7.6; error due to random effects (i.e., block) = 0.52.

Leaf elongation rate (mm/GDD) plotted against cumulative GDD also showed a cubic relationship ($P < 0.001$) for both 300 GDD and 600 GDD plants (Figure 5.3). Maximum leaf elongation rate in the 300 GDD plants was 0.80 mm/GDD compared with 0.66 mm/GDD for 600 GDD plants (Figure 5.3; $P < 0.05$). Leaves of 300 GDD plants reached maximum leaf elongation rate after 185 GDD while 600 GDD plants reached maximum elongation rate after 300 GDD during the regrowth period (Figure 5.3; $P < 0.05$).

Sixteen (67%) of the 24 selected plants from the 600 GDD plots developed stems, while the rest switched to a vegetative state. All 24 selected plants in the 300 GDD plots remained vegetative during the experiment. The time to initiate the elongation of stems was consistent for all replicates at ~274 GDD with a mean stem elongation rate increasing linearly at 1.4 ± 0.8 mm/GDD ($P < 0.01$). Secondary shoots formed along the primary stems at the first three leaf positions, with the first secondary stem visible after 359 GDD.

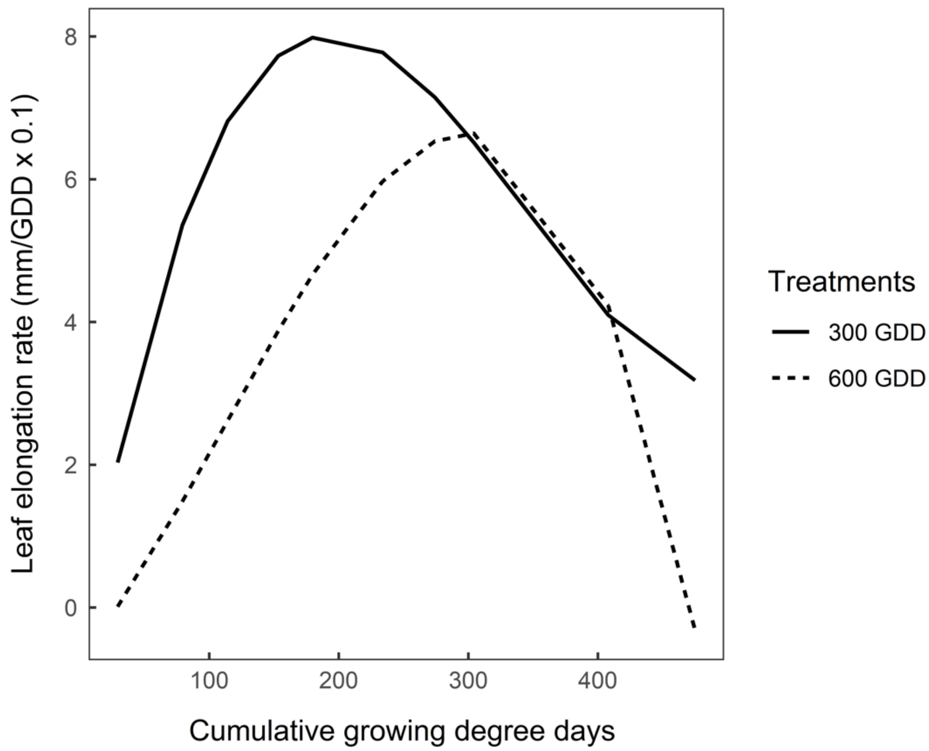

Figure 5.3. Modelled mean leaf elongation rate (mm/GDD × 0.1) of 300 GDD and 600 GDD chicory plants during a regrowth between February and March 2019 (475 GDD). GDD = Growing degree-days. Lines are predicted values plotted against cumulative growing degree-days (GDD) using equations: $y = -0.69 + 0.1x – 0.00037x^2 + 0.00000036x^3$ for 300 GDD plants and $y = -0.69 + 0.02x + 0.0001x^2 – 0.00000031x^3$ for 600 GDD plants ($n = 24$). Root mean square prediction error = 6.24; error due to random effects (i.e., block) = 0.52.

An ordination diagram resulting from the dimensionality reduction analyses of the functional, morphological and physiological traits of vernalised chicory (cv. Choice) at the end of the regrowth period is presented in Figure 5.4. The first two principal components explained 62.9% of the total variability in the data. The ordination diagram revealed a major effect of defoliation interval, where samples of 300 GDD and 600 GDD plots are located at either side of the first axis. The longer defoliation interval was associated with a higher concentration of root WSC, more leaves and secondary shoots per plant, and a greater SPAD value, while the shorter interval was associated with more primary shoots, greater specific leaf area (SLA), and longer leaves (Figure 5.4). The mean values and the associated P – values of the measured traits are presented in Table 5.6.

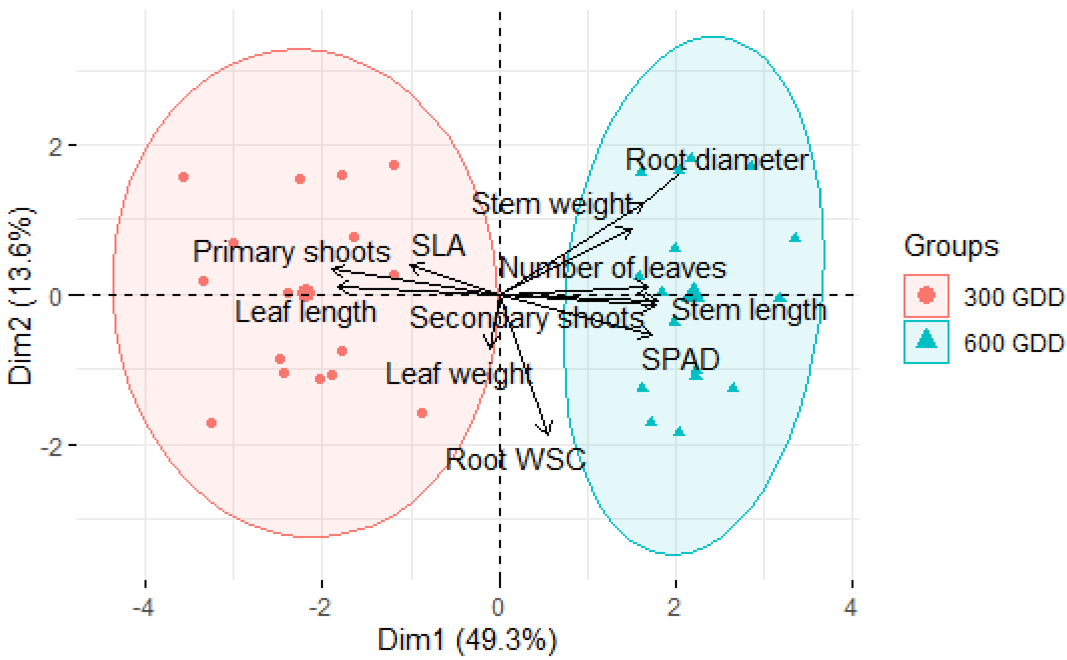

Figure 5.4. Principal Component Analysis biplot showing the variation of functional, morphological and physiological traits of vernalised chicory (cv. Choice) plants under the two defoliation intervals (300 and 600 GDD). WSC = water soluble carbohydrates; SLA = specific leaf area; SPAD = Soil Plant Analysis Development (SPAD) chlorophyll meter; GDD, growing degree-days; Dim1 = dimension 1; Dim2 = dimension 2.

Table 5.6. Number of leaves and shoots per plant, absolute weight of above ground material, root diameter, SPAD and specific leaf area (SLA) of 300 GDD and 600 GDD plants of chicory cv. Choice destructively harvested after a regrowth between February and March 2019.

Item	600 GDD [1]	300 GDD [1]	SEM [5]	*p*-value
Number of live leaves per plant	42.6	28.2	4.13	0.019
Number of primary shoots per plant	2.10	3.79	0.34	0.011
Number of secondary shoots per plant[3]	8.40±0.4	-	-	-
Absolute leaf mass per plant (g DM[2])	2.36	3.05	0.56	0.269
Absolute stem mass (g DM[2]) [3]	2.99±1.2	-	-	-
Final stem lenght (mm) [3]	370±160	-	-	-
Absolute dead material mass (g DM[2])	0.87	0.75	0.11	0.213
Root diameter (mm)	21.4	19.1	1.22	0.272
SPAD[4]	37.5	29.2	1.41	<0.001
Specific leaf area (cm^2/g)	18.6	29.3	2.63	0.007

[1] GDD = Growing degree-days; [2]DM = Dry matter; [3] Data are displayed as arithmetic means ± standard deviation. [4] Soil Plant Analysis Development (SPAD) chlorophyll meter. [5] SEM = Standard error of the mean.

5.4 Discussion

The regrowth intervals chosen in the current experiment were based on observations made by Clapham et al. (Clapham et al., 2001), who reported that chicory cv. Puna bolted at around 400 GDD in southern Western Virginia (USA). With this in mind, we designed the experiment in such a way that plants of chicory cv. Choice would be defoliated to avoid (i.e., 300 GDD) or to encourage (i.e., 600 GDD) bolting, so we could quantify the impact of contrasting defoliation regimes on the responses of individual plants and of the plant community. Our results show interactions between defoliation regimes and the phenological stage of chicory plants, which created quite divergent morphological traits of leaves, shoots and roots and the overall herbage production. Consistent with our expectations, defoliation height had less of an effect (compared with regrowth interval) on most plant functional traits measured on chicory plants, nor on sward features such as plant density and herbage production as has been reported previously (Li et al., 1997c; Labreveux et al., 2004; Lee et al., 2015a). The lack of effect of defoliation height agrees with the observations of Lee et al. (Lee et al., 2015b) who showed that although root reserves of non-structural carbohydrates were depleted following defoliation, they were replenished to pre defoliation levels within 310 GDD, with minimal influence from defoliation to 3 cm or 6 cm residual heights. Therefore, the following discussion is mainly focused on the effects of regrowth interval on morphology and plant functional traits of chicory and on the herbage production, before and after winter vernalisation.

5.4.1 Morphology and functional response of chicory plants

5.4.1.1 Above ground plant morphology of non-vernalised chicory

The size and number of shoots per plant showed the greatest change over time and in response to defoliation regime. After establishment and before vernalisation, chicory plants in all treatments remained vegetative (no bolting stems) and displayed a similar plant architecture with one unique growing point and similar numbers of leaves per plant (Table 5.4). These similarities in plant architecture and phenological stage support previous results reported in New Zealand (Rumball, 1986; Li et al., 1997c) and in USA (Clapham et al., 2001; Labreveux et al., 2004). In developing plants, leaves and roots compete for assimilates, with our results showing the greater sink strength for leaf development, and maintenance of root WSC. Little assimilate was used for shoot initiation which corresponds with Li et al. (Li et al., 1997c). Collectively, these results confirm that the chicory cultivars Choice and Puna require vernalisation to initiate flowering, a process which occurs with low to mid temperatures between 0 °C and 12 °C during winter (Cichota et al., 2020).

5.4.1.2 Above ground plant morphology of vernalised chicory plants

Regardless of defoliation regime (i.e., regrowth interval and defoliation height), all plants increased the number of primary and secondary shoots after winter vernalisation. This response is consistent with Clapham et al. (Clapham et al., 2001) and Li et al. (Li et al., 1997c), who revealed that the initial rosette of chicory split into multi-crowns during the second year on vernalised plants. The PCA results show the degree of morphological separation between the two defoliation frequencies (Figure 5.4). Frequently defoliated chicory plants (300 GDD) produced more primary shoots and of smaller size, while 600 GDD plants produced fewer but bigger primary shoots, which held more secondary shoots (Table 5.4). The primary shoots in 300 GDD plants were mostly vegetative material, while those formed on 600 GDD plants were mostly reproductive, with only occasional vegetative shoots. Li et al. (Li et al., 1994) also reported a flush of vegetative shoots during the growing season in chicory plants grazed every 3–5 weeks, an interval equivalent to the

300 GDD interval in the current experiment. Following defoliation, plants produce more vegetative growth to increase their photosynthetic capacity and enhance growth rate to compensate for the lost biomass (McNaughton, 1983; Briske and Richards, 1995) and to replenish root reserves (Lee et al., 2015b). There are two plausible explanations for the decreased number of primary shoots in 600 GDD plants; firstly, this can be due to an auxin-induced growth of a main stem that inhibits the outgrowth of lateral crown buds by diverting sugars away from the bud (Kebrom, 2017). Frequent defoliation might remove the apical domination of the main stem and thus remove the growth inhibition of other crown buds allowing lateral crown shoots to be relatively further advanced before one shoot became dominant (Leach, 1979). The other explanation is related to a shading effect, as young vegetative shoots of 600 GDD plants likely died because of lack of sufficient light to maintain growth as taller reproductive shoots capture most radiation (Smith and Leinweber, 1973; Ong, 1978). Even though our results showed an increased photosynthetic capacity of leaves in 600 GDD plants (higher SPAD values), it might not have been sufficient to compensate for the reduced radiation interception by lateral shoots for sustaining growth which therefore resulted in premature senescence of lower leaves.

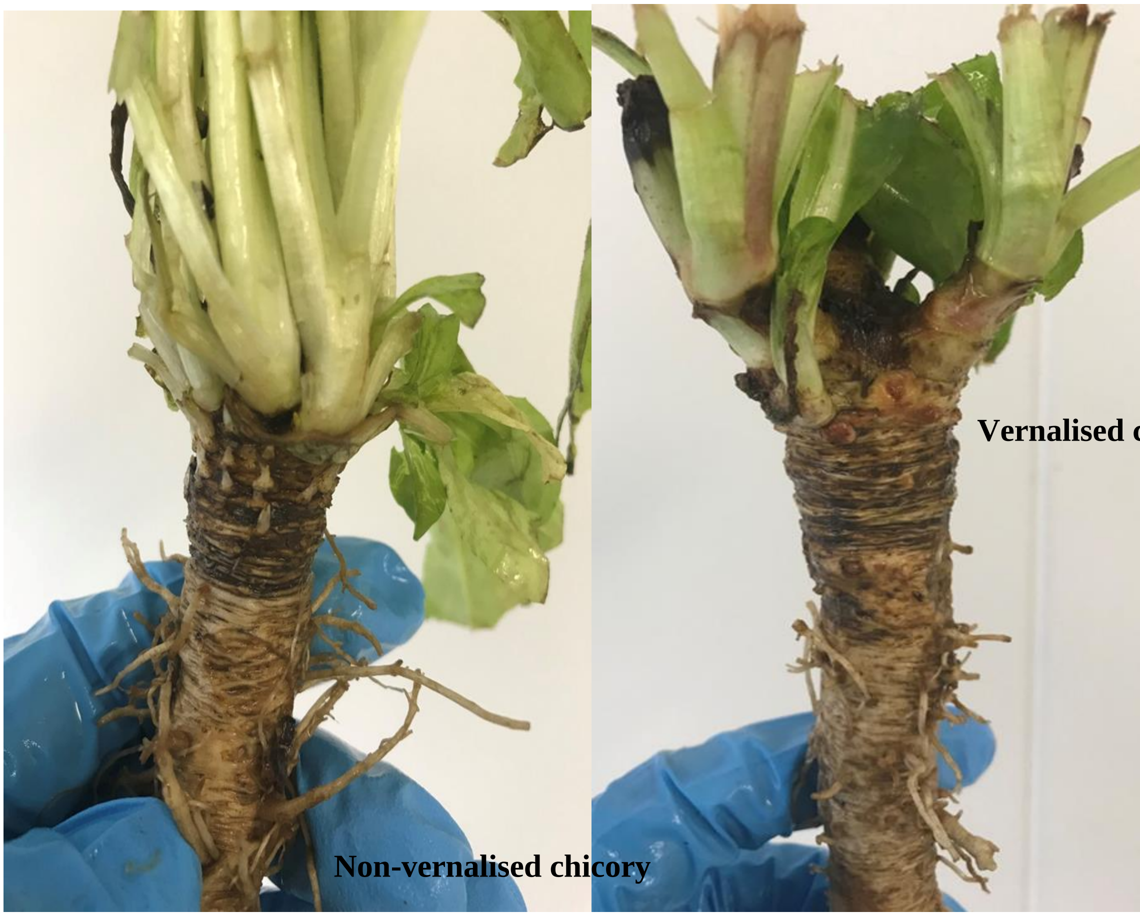

Plate 5.5. Vernalised and non-vernalised chicory plants. The non-vernalised chicory plants had 1-2 unique growing point (crown). The initial rosette of chicory split into multi-crowns during the second year on vernalised plants.

The increase in number of shoots after vernalisation was associated with the corresponding increased biomass of chicory plants after vernalisation (Table 5.4). When comparing the two regrowth intervals, individual plant biomass was greater for 600 GDD plants than 300 GDD plants. Generally, plants (grasses, legumes or herbs) that have reached reproductive stage are more productive in terms of individual plant biomass (Li et al., 1997c; Solomon et al., 2017; Ta et al., 2020). The increased plant weight for 600 GDD plants was related to stem weight. On a dry weight basis, stem accounted for 55% and 31% of the respective biomass for 600 GDD and 300 GDD plants after vernalisation. The proportion of stem observed here corroborates previous research showing a 15% to 50% range for chicory stem (Rumball, 1986; Li et al., 1997c; Clapham et al., 2001). Similarly, for the vegetative 300 GDD plants, the mean plant weights (2.34 g and 4.86 g of DM per plant before and after vernalisation, respectively) are not dissimilar to the 3.86 g of DM per plant reported by Li et al. (Li et al., 1997c).

Despite the greater leaf number in 600 GDD plants after vernalisation, the absolute leaf mass per plant was not different between treatments (Table 5.4). This indicates the greater leaf weight of 300 GDD plants compared with 600 GDD plants, which is explained by longer leaves (Figure 5.1) with greater specific leaf area (Table 5.6) than the leaves of 600 GDD plants. Leaf size largely depends on plant phenology, with a progressive decrease in leaf size as the plant transitions from vegetative to reproductive stages (Doorenbos and Riemens, 1959; Clapham et al., 2001). As plants grow taller and prepare to reproduce, they partition most of their carbon to the development of stems (at the expense of leaves) to maintain an erect position to display their leaves and reproductive structures (flowers) within the well exposed layers of the canopy (Vallet et al., 1998; Ta et al., 2020). However, the leaf is the most important photosynthetic organ of plants and an important parameter in determining plant productivity (Shi et al., 2019). Therefore, to cater for the increased plant demand, the small leaves from 600 GDD plants increased their photosynthetic capacity as shown by their higher SPAD values (Table 5.6).

5.4.2 Root size and concentration of stored carbohydrates

The taproot of chicory is the main storage organ of WSC used for plant growth after defoliation or for winter dormancy (Monti et al., 2006; Lee et al., 2015b). Following defoliation, the leaf photosynthetic rate increases and the actively growing points in plants become high priority sinks of the currently produced photosynthetic carbon and the reserves located in the roots (Briske and Richards, 1995; Holland et al., 1996). Consequently, the root size and concentrations of WSC tend to be lower in more frequently grazed plants of *Lolium perenne* L. than in less frequently grazed ones (Donaghy and Fulkerson, 1997; Solomon et al., 2017). Our results showed that both the size of the roots and their concentration of WSC were lower for the 300 GDD plants than the 600 GDD plants before and after vernalisation. Similarly, Donaghy and Fulkerson (Donaghy and Fulkerson, 1998) and Solomon et al. (Solomon et al., 2017) have reported a decline in root WSC concentration from longer to shorter defoliation intervals in perennial and annual ryegrass plants, respectively.

In addition to a regrowth interval effect, we also observed a phenological stage effect on the root WSC concentration. Vernalised plants had 46% and 35% lower concentration of root WSC than non-vernalised plants under 300 GDD and 600 GDD regrowth intervals, respectively. This is probably because chicory is a winter dormant plant, which utilises stored WCS for its survival during winter (Li et al., 1997c; Li and Kemp, 2005). The plant is also transitioning to a reproductive stage after vernalisation, which requires greater energy resources (Ong, 1978).

5.4.3 Herbage production and plant density of chicory swards

5.4.3.1 Before vernalisation

Before vernalisation between February and May 2018, all chicory treatments accumulated on average 6.4 ± 0.98 kg/ha DM herbage per GDD. Variation in accumulated herbage mass in response to contrasting regrowth intervals of the same forage species likely result from differences in plant density and plant size (Gastal and Lemaire, 2015; Solomon et al., 2017). In the current experiment, no significant differences in plant density between treatments were found (Table 5.5). Additionally, all plants were in a rosette growth form with a similar plant structure, suggesting that 300 GDD chicory plants increased their growth rate after a grazing event to compensate for the lost biomass and subsequently accumulated equal yield as 600 GDD plants before vernalisation (McNaughton, 1983).

5.4.3.2 After vernalisation

After winter vernalisation, between October 2018 and February 2019, 600 GDD plants accumulated greater herbage mass than 300 GDD plants (8.7 vs 7.7 ± 0.49 kg DM/ha per GDD). Several other studies have used a fixed number of calendar days or plant height as a criterion for when to defoliate chicory (Li et al., 1997a; Labreveux et al., 2004; Lee et al., 2015a). Regardless of which defoliation criterion was used in these studies, chicory plants defoliated after longer intervals accumulated greater herbage mass than those defoliated after shorter intervals. The total accumulated mass of vernalised chicory plants harvested every 300 GDD between October 2018 and February 2019 was consistent with the yield range of 9.6 t DM/ha – 11.2 t DM/ha reported for second year chicory defoliated at 3–5 weeks in spring and summer in New Zealand (Li et al., 1997a; Lee et al., 2015a). As plant density was not different between treatments, the differences among treatments in plant structure likely explains the differences in herbage production between the treatments after vernalisation. For example, 600 GDD plants had more stem material than 300 GDD plants. The rapid growth rate of the reproductive stem of vernalised 600 GDD plants (1.4 mm/GDD) might have increased the overall growth rate, subsequently accumulating more herbage mass than 300 GDD plants, which remained vegetative. Moreover, the greater concentration of WSC in roots of 600 GDD plants may explain their increased herbage mass compared with 300 GDD plants. Stored carbohydrates play a vital role in the growth and development of forages in temperate regions and their increased concentration is associated with greater forage yield (Solomon et al., 2017).

After vernalisation, a decline in plant density of chicory is expected, regardless of defoliation regime (Li et al., 1997a; Clapham et al., 2001; Li and Kemp, 2005). With continuous defoliation, the root size and stored carbohydrates decrease, and the plants eventually die due to starvation, as has been shown for chicory and other temperate tap rooted forages such as red clover (Kendall, 1958; Li et al., 1997c). Another possible reason for the decline in plant density in chicory is treading damage of new buds on the crown and the crown itself, more especially when grazed by dairy cows (Li and Kemp, 2005). Plants tend to increase their individual weight and that way, may compensate the loss of plants, maintaining forage mass (Leach, 1979; Li et al., 1997c), as was also observed in the current experiment. However, as Leach (1979) reported, “the inevitable loss of plants will subsequently decrease yields per unit area in the long run”. Under temperate conditions in New Zealand, a decline in chicory yield was observed after four years when the density was less than 25 plants/m^2 (Li and Kemp, 2005).

5.4.4 Implications

In forages, there is a potential trade-off between herbage production and feeding value as plants exposed to longer regrowth intervals accumulate larger amounts of aerial mass, and reduce the palatable and the highly nutritious leaf proportion of the herbage (Ta et al., 2020). In the current experiment, the time to the initiation of stem elongation for vernalised chicory cv. Choice was ~274 GDD. By grazing chicory every 300 GDD, we were able to control the growth and development of the mature stem material. Plants grazed at longer intervals (e.g., 600 GDD) had higher stem-to-leaf ratio suggesting that the herbage mass of such swards have reduced feeding value. However, our results showed that although 600 GDD plants produced more stem than leaf material, the absolute leaf mass did not differ from those plants grazed at 300 GDD intervals. This is consistent with the results by Clapham et al. (2001), who also reported no differences in total leaf area between vegetative and reproductive plants of vernalised chicory. Therefore, appropriate herbage allocation of vernalised chicory herbage to livestock would need to account for stem refusal in reproductive plants, to avoid underfeeding and allow diet selection for digestible biomass to achieve high livestock performance.

Finally, the consequence of low-quality mature stems on animal performance should be considered. Hunt and Hay (1990) and McCoy et al. (1997) reported that ruminants preferred plants that have not bolted. Barry (1998) recommended that chicory should be grazed before bolting to maintain plants in the vegetative stage. Based on Barry (1998) and the results from the current experiment, chicory plants should be grazed after a minimum of 4 weeks in autumn, and 3-4 weeks in spring and summer in order to control growth of reproductive stems. This period will also coincide with the peak leaf elongation rate (185–300 GDD; Figure 5.3), meaning grazing animals would capture the large, fully developed basal leaves of the plants before they senesce (Clapham et al., 2001). Extending the autumn regrowth interval similar to 6 weeks may aid recovery of root reserves, as seen in 600 GDD treatments and potentially improve plant longevity.

5.5 Conclusions

Under the conditions of the current experiment, plants of chicory cv. Choice managed under shorter regrowth intervals (i.e., 300 GDD) remained mostly vegetative with heavier and longer leaves, though with reduced photosynthetic capacity, than 600 GDD plants. Moreover, 300 GDD plants had narrower root diameter and lower root WSC, which is likely to compromise longevity of the forage crop. To maintain a vegetative, high feed quality forage crop after vernalisation, a more frequent defoliation regime is recommended, though the resulting decline in root WSC reserves is likely to compromise its longevity. Opportunity exists for a combination of frequent and infrequent defoliation regimes to optimise both vegetative growth and root reserves, and the effect of seasonal defoliation frequencies is an area for further exploration.

Chapter 6

6. Effect of vernalisation on the diurnal changes in fatty acid profile and nutrient composition of chicory in response to defoliation frequency

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Statement of Contributions of Joint Authorship

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6.1 Introduction

Milk quality from pastoral grazed systems is becoming increasingly important, driven mainly by the increased concentration of health promoting fatty acids (**FA**) in the milk. Perennial ryegrass (**PR**; *Lolium perenne*) is the pivotal pasture species in the pastoral grazed system in temperate regions such as New Zealand. Freshly grazed PR contains greater concentrations of polyunsaturated FA (Sun & Gibbs, 2012; Rugoho et al., 2017), increasing the concentration of Omega-3 FA and conjugated linoleic acid (**CLA**; C18:2 c9, t11) in the milk when compared with silage-based or concentrate-based diets (Elgersma, 2015). Linoleic acid (**LA**; C18:2 c9, c12) and α-linolenic acid (ALA; C18:3 c9, c12, c15) in the herbage are the two main precursors of these beneficial FA in ruminant products (Lock & Bauman, 2004). While diet LA and ALA are extensively biohydrogenated in the rumen, it has been shown that increasing their concentration in the diet of dairy cows can substantially improve both their concentration and that of CLA in ruminant-source foods (Elgersma et al., 2006).

At the farm level, it is possible to increase the concentration of desirable FA in milk of dairy cows through the inclusion of novel forage herbs into the traditional diet of PR (Mangwe et al., 2020). Alternative forages such as chicory have frequently been found to contain greater concentrations of PUFA in the herbage when compared with PR. In Switzerland, Puna chicory herbage sown with ryegrass (at 80% chicory and 20% ryegrass) contained 36% higher concentrations of LA than pure swards of ryegrass (Kälber et al., 2011). In southwest Victoria, Australia, an average of 5.38 mg/g of DM of LA was obtained in pure swards of chicory during the spring, which was greater than the average of 2.10 mg/g of DM for PR (Muir et al., 2014). In the same experiment, ALA did not differ between chicory and PR herbages (Muir et al., 2014). However, in summer Muir et al. (2015) found higher ALA concentrations in chicory herbage than PR herbage. Recent studies show that chicory feeding can improve the concentration of CLA (Muir et al. ,2015; Rodríguez et al., 2020) and ALA in meat and milk (Muir et al., 2014; Mangwe et al., 2020a, b) of ruminants when compared with PR. The composition of FA in the herbage is driven as much by forage type as by management practices (Dewhurst et al., 2006; Lourenço et al., 2008). Information available in the literature show that the levels of LA and ALA in PR herbage varies considerably, depending on growth stage and defoliation interval. Growth stage and defoliation interval affect the morphology of the crop and therefore, the herbage FA composition (Dewhurst et al., 2001; Elgersma et al., 2003a).

While these changes are well documented for PR, studies aimed at quantifying and describing such trends in chicory forage are lacking.

Grazing management practices including defoliation interval and severity have been identified as major factors influencing the nutritive composition of forages in pastoral grazed systems. Several studies have investigated grazing interval for chicory in terms of nutritive composition (Clark et al., 1990; Li et al., 1997; Labreveux et al., 2004; Lee et al., 2015), though the criterion used to determine when to defoliate chicory in all these studies was based on calendar days or sward surface height. Fixed number of calendar days does not consider climatic variation between seasons and locations that will affect both the stage of regrowth and amount of herbage present at the end of each specified period (Fulkerson & Donaghy, 2001). Our earlier research demonstrated that using thermal time, and including vernalisation as a factor, to assess morphological response of chicory to defoliation management enabled us to better predict the plant growth response (Mangwe et al., 2020c). In that study the large variation in leaf and stem development, at advancing phenological stage, highlighted the potential impacts of post-vernalisation management on nutrient composition and subsequent animal response when grazed in situ.

Given the growing interest in the use of forage herbs to curb the environmental challenges associated with livestock systems based on the traditional PR (Carlton et al., 2019; McCarthy et al., 2020), consideration of protein content and fermentable carbohydrates are increasingly important components of the nutritive characteristics of the plant. In a review, Edwards et al. (2007), reported that increases in readily fermentable carbohydrates to crude protein (**CP**) ratio could improve nitrogen (**N**) use efficiency, reduce concentration of N in the urine, diminishing the environmental impacts associated with pastoral livestock systems. Afternoon herbage of herbaceous plants is characterized with increased concentration of readily fermentable carbohydrates at the expense of CP (Delagarde et al., 2000; Bryant et al., 2014). Knowledge of the diurnal variations in nutrient characteristics of chicory could help design grazing management practices that will further diminish the environmental impacts associated with grazed pastoral systems, while enhancing the concentration of the health promoting FA in ruminant-source foods. The objective of this study was to compare the effects of defoliation frequency and severity on FA concentration and nutrient composition at different times of the day before and after vernalisation.

6.2 Materials and methods

6.2.1 Experimental site and design

Monocultures of chicory cv. Choice were sown at 5.3 kg/ha in four 1.5 ha paddocks at the Lincoln University Research Dairy Farm, Canterbury, New Zealand (43^{o} 38` S, 172$^{o}$ 28` E; 17 m above sea level) in November 2017. The study was a randomized block design with four blocks (4 paddocks). A small portion in each of the four paddocks was permanently fenced off and sub-divided into four 10 m × 10 m plots adjacent to each other and treatments randomly assigned within those areas. Treatments included two regrowth intervals and two defoliation heights. The two regrowth intervals were 300 and 600 growing degree days (GDD), and the two defoliation heights were 4 and 8 cm stubble height. Regrowth intervals in GDD were based on cumulative GDD, calculated above a 4 ^{o}C base temperature as: GDD = ((daily maximum temperature + daily minimum temperature)/2) – 4)). The temperature was set to equal 4 ^{o}C when the mean daily temperature was below 4 ^{o}C (McMaster & Wilhelm 1997). Details of establishment and soil type of the present study are given in Chapter 5.

6.2.2 Management

The present study took place over 51 weeks between 15 February 2018 and 05 February 2019. From sowing in November 2017 to 16 February 2018, the stand of chicory in all four blocks were grazed twice, with first grazing on 16 January 2018. The second grazing was on 15 February 2018 to prepare the area for the present study. A small portion in each of the four paddocks was permanently fenced off and sub-divided into four 10 m × 10 m plots adjacent to each other and treatments randomly assigned within those areas. Climatic data and irrigation details are given in Chapter 5. Nitrogen fertiliser was applied at three occasions during the study: at 30, 50, and 30 kg N/ha in February, October, and December 2018 following grazing all plots.

During the growing season (September to May), chicory plots was grazed by 3-6 dairy cows (484 ± 16.9 kg BW) when the target GDD had been reached. As per standard practise chicory was not grazed during winter because of sensitivity to crown damage by treading and low growth rate (Li and Kemp, 2005). In order to achieve target defoliation height at each grazing event, the grazing duration was extended for the 4 cm stubble height treatment.

6.2.3 Herbage sampling

Herbage samples were collected over two consecutive days prior to grazing (Table 6.1). Herbage was sampled twice on each day at 0630 hrs and at 1600 hrs, to simulate either a morning or afternoon allocation of fresh herbage to livestock. At each sampling, herbage samples were collected by cutting with scissors at respective defoliating height (4 and 8 cm) above ground level in six random locations in each plot. Samples were homogenized and separated into thirds to determine dry matter percentage (**DM**%), chemical and botanical components. The DM% was determined by oven drying at 60 ^{o}C for 48 hrs. Botanical components were separated as chicory, PR, weed or dead material. Chicory herbage was further separated into leaves or reproductive stems. All components were oven dried at 60 ^{o}C for 48 hrs to determine their percentage contribution in the stand. Samples for chemical composition and FA acid analysis were frozen and stored at 20 ^{o}C before being freeze dried and ground to pass through a 1mm sieve (ZM200 Retsch GmbH; Haan, Germany).

Table 6.1. Date of defoliation, date of herbage sampling, and regrowth age [accumulated days and growing degree-days (GDD)] between defoliation events.

Date of defoliation	Treatment	Regrowth age	
		Days	Cumulative GDD
15-Feb-18	300 GDD		
15-Feb-18	600 GDD		
26-Mar-18	300 GDD	38	480
6-Apr-18	600 GDD	49	609
28-Apr-18	300 GDD	33	298
Vernalisation (June & July 2018)			
5-Oct-18	300 GDD	161	740
5-Oct-18	600 GDD	183	907
13-Nov-18	300 GDD	38	320
13-Dec-18	300 GDD	30	298
13-Dec-18	600 GDD	69	636
8-Jan-19	300 GDD	26	313
5-Feb-19	300 GDD	22	402
5-Feb-19	600 GDD	49	715

6.2.4 Chemical composition and FA analysis

Samples were scanned for CP, organic matter (**OM**), soluble sugars and starch (**CHO**), acid and neutral detergent fibre (**ADF** and **NDF**), digestible organic matter content (**DOMD**) using near infrared spectrophotometry (**NIRS**. Model: FOSS NIRS Systems 5000, Maryland USA). Calibration equations for CP (Variomax CN Analyser, Elementar), CHO (MAFF, 1986), NDF

(Van Soest et al., 1991) and ADF (method 973.18; AOAC, 1990), DOMD and DMD (Iowerth et al., 1975) were previously derived on chicory and plantain forages. The R-squared values for all the parameters were above 0.9 and all samples in the present study fell within the calibration range. Metabolisable energy (**ME**) content was estimated based on digestibility as; ME (MJ/kg DM) = 0.16 * DOMD (CSIRO, 2007). Non-fibre carbohydrates (**NFC**) were estimated as (100- (NDF + CP + Fat + Ash)). Samples for FA analysis were prepared by transmethylation and analysed by gas chromatography (with AOC-20i auto-sampler, Shimadzu GC-2010, Japan), according to AOAC (2012) Method 2012.13 using a Varian CP742 silica capillary column (0.25 × 100 m × 0.2 µm).

6.2.5 Statistics

Chemical components and FA composition of the herbage were analysed using mixed effect models with the lme function of the 'lme4' package version 1.1–21 of R (Pinheiro et al., 2018). Plot was the experimental unit, regrowth interval (300 GDD and 600 GDD), defoliation height (4 cm and 8 cm), diurnal (morning and afternoon), phenological growth stage (before and after vernalisation) and their interactions were considered as fixed effects and block and season as nested random effects. Mean separation was done using the 'emmeans' package version 1.4.5 of R, with Tukey's method for comparing the estimates. Differences were declared significant if $P \leq 0.05$ and tendencies if $P \leq 0.1$.

A principal component analysis (**PCA**) was used to summarize the variation of FA and nutrient composition of chicory herbage managed under the two regrowth intervals. Dimensionality reduction analyses were conducted using the 'factoextra' package, version 1.0.7 of R (Kassambara and Mundt, 2020), with regrowth interval as explanatory variables and FA and nutrient composition as response variables. The analysis were conducted for vernalised and non-vernalised chicory separately and together.

6.3 Results

All stands were dominated by chicory, containing less than 5% of unsown species before vernalisation. After vernalisation, the herbage contained (of DM) 85% chicory, 8% PR, 5% broad-leaved weeds and 2% dead material. There was an interaction ($P < 0.001$) between vernalisation and regrowth interval for reproductive stem material. Chicory herbage was vegetative before vernalisation, with no reproductive stem. While after vernalisation, the reproductive stem material was greater in 600 GDD compared with 300 GDD, accounting for 64.1% and 59.8% of DM in 600 GDD, and for 41.9% and 28.6% of DM in 300 GDD herbage in December 2018 and February 2019, respectively.

6.3.1 Fatty acid profile

The significance of treatment effects and mean of individual FA and total FA of chicory herbage before and after vernalisation is presented in Tables 6.2 and 6.33. The effect of defoliation height and its interactions was not significant for most of the FA (Table 2). The concentration of individual FA and total FA exhibited rather little variation with regrowth interval, defoliation height and time of day before the plants were vernalised (Table 3).

Palmitic acid (C16:0), LA and ALA, were the predominant FA in chicory herbage, accounting for 93 - 96% of the total FA before and after vernalisation. Palmitoleic acid (C16:1 c9), stearic acid (C18:0) oleic acid (C18:1 c9) and lignoceric acid (C24:0) were the next most abundant FA before vernalisation, stearic and oleic acids were also the next most abundant FAs after vernalisation, accounting for an average total of 2.5% of the total FA measured across treatments and phenological stages. The remaining FAs were present at very low concentrations (< 0.2 mg/g DM) in the herbage.

On average, the concentration of palmitic acid, LA, ALA and total FA in the herbage was, respectively, 6.3, 10.9, 32.7 and 52.2 mg/g DM across treatments before vernalisation. Differences were only detected for oleic acid concentration ($P < 0.01$) between morning and afternoon herbage, with the concentration of oleic acid being 23% higher in the afternoon than the morning across regrowth intervals and defoliation heights (Table 3).

Vernalisation of chicory resulted in a reduction in all FA concentrations ($P \leq 0.023$), except for oleic acid ($P = 0.79$). The magnitude of decline with progressing development stage was different for each FA. For example, the decline in total FA after vernalisation ranged from 56% for 300 GDD to 70% for 600 GDD herbage. Consequently, total FA concentration was 49% greater for 300 GDD herbage than 600 GDD herbage after vernalisation (52.2 vs 19.3 mg/g DM; $P < 0.01$). Total FA concentration was 12% higher in the afternoon than in the morning, but the differences were not significant ($P = 0.133$).

As with total FA, the ALA concentration declined during phenological development, and the decrease in herbage was more pronounced after 600 GDD compared with after 300 GDD. After vernalisation the ALA concentration of 300 GDD herbage always surpassed concentrations found in 600 GDD herbage (10.8 mg/g DM vs. 6.5 mg/g DM), regardless of defoliation height or time of day. Differences were also detected for LA concentration between the two regrowth intervals after vernalisation, with 300 GDD herbage having higher values than 600 GDD herbage (6.3 mg/g vs 4.5 mg/g DM; P = 001). Several FA were sensitive to diurnal changes including LA which had greater herbage concentrations in afternoon than in the morning (P = 0.035), and differences in LA concentration between morning and afternoon herbage being larger in 300 GDD (+23%) than in 600 GDD (+17%). The pattern was the same for oleic acid; 37% higher in 300 GDD than 600 GDD herbage after vernalisation, with 300 GDD afternoon herbage having a 24% further increment in oleic acid concentration (P < 0.001) compared with 300 GDD morning herbage.

Table 6.2. F probability of effects of regrowth interval (R), defoliation height (H), diurnal pattern (D) and vernalisation (V), and interaction effects on fatty acid profile of chicory cv. Choice.

Fatty acids	**Regrowth**	**Diurnal**	**Height**	**Vernalisation**	**R× H**	**R × V**	**D × V**	**H × V**
C14:0	0.001	0.439	0.512	0.002	0.478	0.013	0.212	0.888
C15:0	0.042	0.018	0.239	<0.001	0.737	0.184	0.836	0.493
C16:0	0.001	0.087	0.545	<0.001	0.677	0.001	0.472	0.892
C16:1 c9	0.007	0.138	0.083	<0.001	0.001	0.257	0.230	0.105
C17:0	0.834	0.047	0.195	<0.001	0.370	0.658	0.991	0.722
C18:0	<0.001	0.061	0.172	<0.001	0.739	0.015	0.548	0.391
C18:1 c9	0.010	<0.001	0.893	0.789	0.909	0.001	0.166	0.591
C18:2 c9,12	0.001	0.035	0.757	<0.001	0.717	0.001	0.347	0.753
C18:3 c9,12,15	<0.001	0.384	0.137	<0.001	0.031	0.004	0.451	0.272
C20:0	0.018	0.469	0.374	<0.001	0.983	0.765	0.240	0.845
C22:0	0.009	0.595	0.818	<0.001	0.456	0.058	0.393	0.746
C23:0	0.345	0.920	0.197	0.023	0.209	0.220	0.671	0.835
C22:2 c13,16	0.060	0.260	0.284	<0.001	0.528	0.301	0.016	0.35
C24:0	<0.001	0.150	0.679	<0.001	0.242	0.019	0.070	0.625
Total	<0.001	0.133	0.291	<0.001	0.157	0.001	0.417	0.615

Table 6.3. Effects of regrowth interval, defoliation height, time of day and vernalisation, and interaction effects on fatty acid (mg/g DM) profile of chicory cv. Choice.

Interval	**300 GDD**				**600 GDD**				
Height	**4 cm**		**8 cm**		**4 cm**		**8 cm**		**SEM**
Time	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	
Before vernalisation									
C14:0	0.10	0.09	0.10	0.09	0.09	0.09	0.10	0.09	0.010
C15:0	0.10	0.10	0.10	0.10	0.09	0.11	0.10	0.10	0.007
C16:0	6.42	6.24	6.34	6.35	5.86	6.66	6.35	6.51	0.508
C16:1 c9	0.71	0.65	0.63	0.66	0.52	0.63	0.67	0.69	0.022
C17:0	0.06	0.06	0.06	0.06	0.05	0.06	0.05	0.06	0.004
C18:0	0.32	0.30	0.33	0.33	0.27	0.31	0.30	0.33	0.024
C18:1 c9	0.27^{b}	0.29^{b}	0.27^{b}	0.37^{a}	0.35^{ab}	0.41^{a}	0.27^{b}	0.36^{a}	0.044
C18:2 c9,12	10.2	11.1	10.8	10.7	10.7	11.5	10.8	11.2	0.648
C18:3 c9,12,15	32.4	34.8	32.8	33.0	28.7	32.1	33.3	34.3	1.340
C20:0	0.13	0.12	0.13	0.13	0.12	0.12	0.12	0.12	0.07
C22:0	0.17	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.012
C23:0	0.08	0.08	0.08	0.08	0.08	0.07	0.08	0.08	0.006
C22:2 c13,16	0.10	0.09	0.10	0.09	0.08	0.09	0.10	0.09	0.007
C24:0	0.32	0.29	0.30	0.29	0.29	0.27	0.31	0.28	0.018
Total	51.4	54.3	52.2	52.4	47.4	52.6	52.6	54.4	2.460
After vernalisation									
C14:0	0.07	0.08	0.07	0.08	0.05	0.05	0.05	0.06	0.008
C15:0	0.04b	0.06a	0.05b	0.05b	0.06a	0.05b	0.06a	0.07a	0.006
C16:0	4.21^{b}	5.28^{a}	4.45^{b}	5.15^{a}	3.52^{c}	3.17^{c}	3.32^{c}	3.83^{c}	0.390
C16:1 c9	0.04	0.04	0.04	0.04	0.03	0.02	0.02	0.03	0.017
C17:0	0.02^{b}	0.03^{a}	0.02^{b}	0.03^{a}	0.03^{a}	0.02^{b}	0.03^{a}	0.03^{a}	0.003
C18:0	0.20^{b}	0.24^{a}	0.20^{b}	0.24^{a}	0.15^{c}	0.15^{c}	0.15^{c}	0.17^{c}	0.018
C18:1 c9	0.31^{b}	0.43^{a}	0.34^{b}	0.38^{ab}	0.26^{c}	0.24^{c}	0.26^{c}	0.28^{c}	0.034
C18:2 c9,12	5.52^{b}	7.04^{a}	5.88^{b}	6.89^{a}	4.17^{c}	4.67^{c}	4.14^{c}	5.13^{bc}	0.515
C18:3 c9,12,15	10.1^{b}	11.40^{a}	9.81^{b}	11.80^{a}	6.30^{c}	6.30^{c}	6.60^{c}	6.79^{c}	0.990
C20:0	0.07	0.07	0.07	0.08	0.06	0.06	0.06	0.07	0.005
C22:0	0.13	0.14	0.12	0.14	0.11	0.11	0.11	0.12	0.009
C23:0	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.07	0.005
C22:2 c13,16	0.02	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.005
C24:0	0.17	0.18	0.15	0.18	0.14	0.12	0.13	0.13	0.013
Total	20.1^{a}	25.1^{a}	21.3^{a}	25.1^{a}	14.8^{b}	15.2^{b}	15.1^{b}	16.8^{b}	1.84^{b}

$^{a–c}$ Means within a row with different superscript letters differ significantly ($P < 0.05$).

6.3.2 Nutrient composition

The significance of treatment effects and mean of nutrient components of chicory herbage before and after vernalisation is presented in Tables 6.4 and 6.5. Interactions occurred between vernalisation and regrowth for CP showing that the average CP concentration of the herbage before vernalisation was 198 g/kg DM and was unaffected by regrowth interval. By contrast, after vernalisation the average CP concentration declined to 119 or 84 g/kg DM at 300 and 600 GDD respectively (Table 6.5). Before vernalization, afternoon herbage had a lower CP concentration than morning herbage ($P < 0.001$), with a mean difference of 15.5 g/kg DM across intervals (Table 6.5). The differences in CP concentration between morning and afternoon after vernalisation was greater in 300 GDD (17 g/kg DM) than in 600 GDD herbage (10 g/kg DM; interaction interval × vernalisation × time of day $P = 0.042$).

On average, the CHO concentration of the herbage ranged from 139 g/kg DM for 300 GDD to 149 g/kg DM 600 GDD herbage across phenological stages. Differences were detected for CHO concentration between morning and afternoon herbage before and after vernalisation. Before vernalisation, CHO concentration of afternoon herbage surpassed concentrations found in morning herbage by 21.7%. After vernalisation, CHO concentration of afternoon herbage surpassed concentrations found in morning herbage by 15% in 300 GDD and by 9.3% in 600 GDD herbage. The CHO:CP ratio was increased by harvesting in the afternoon, regardless of defoliation height and phenological stages (Table 6.5).

The average NDF concentration was 166 g/kg DM before vernalisation and 343 g/kg DM after vernalisation ($P < 0.001$; Table 5). Differences were detected for NDF concentration between the two regrowth intervals after vernalisation ($P < 0.001$), with 600 GDD herbage having 68% greater NDF concentration than 300 GDD (422 vs 260 g/kg DM). The NDF concentration decreased during the day across phenological stages, although the variation was greater in 300 GDD (12.9%) herbage compared with 600 GDD (4.8%) herbage after vernalisation (interaction interval × vernalisation × time of day $P = 0.012$). The ADF concentration was higher in vernalised than non-vernalised chicory plants. Chicory herbage under longer regrowth intervals (600 GDD) contained 42% greater ADF concentration compared with that under shorter regrowth intervals (300 GDD).

Generally, DOMD and ME were greater prior to vernalisation as a higher stem proportion following vernalisation increased the fibre fractions and lowered digestibility. However, a regrowth interval by vernalisation interaction ($P < 0.001$) for DOMD revealed that before vernalisation, there were no effects of regrowth interval on DOMD (ranged from 813 to 855 g/kg DM; Table 6.5) but after vernalisation DOMD declined with increased regrowth interval ($P < 0.001$). There was a tendency for diurnal fluctuations after vernalisation ($P = 0.08$), with afternoon herbage having 3.2% greater DOMD than morning herbage.

Table 6.4. F-probability of effects of regrowth interval (I), defoliation height (H), time of day (T) and vernalisation (V), and interaction effects on chemical composition of chicory cv. Choice.

Item	**Regrowth**	**Diurnal**	**Height**	**Vernalisation**	**R × D**	**R× V**	**V × D**	**H × V**
Dry matter	< 0.001	0.011	0.145	0.001	0.222	0.002	0.552	0.555
Organic matter	< 0.001	0.269	0.149	0.134	0.747	< 0.001	0.680	0.870
Crude protein	< 0.001	0.013	0.740	< 0.001	0.098	< 0.001	0.001	0.339
Soluble sugars and starch	0.148	< 0.001	0.109	< 0.001	0.062	0.001	< 0.001	< 0.001
Non fibre carbohydrates	< 0.001	0.009	0.095	< 0.001	0.559	< 0.001	0.293	0.002
Neutral detergent fibre	< 0.001	0.019	0.205	< 0.001	0.893	< 0.001	0.685	0.149
Acid detergent fibre	< 0.001	0.838	0.295	< 0.001	0.984	< 0.001	0.047	0.081
DOMD	< 0.001	0.080	0.151	< 0.001	0.495	< 0.001	0.318	0.028
Metabolisable energy	< 0.001	0.080	0.151	< 0.001	0.495	< 0.001	0.318	0.028

DOMD; Digestibility of the organic matter in the dry matter.

Table 6.5. Effects of regrowth interval (I), defoliation height (H), time of day (T) and vernalisation (V), and interaction effects on chemical composition of chicory cv. Choice.

Interval	300 GDD				600 GDD				SEM
Height	4 cm		8 cm		4 cm		8 cm		
Time	AM	PM	AM	PM	AM	PM	AM	PM	
Before Vernalisation									
DM (% of FW)	9.22^{c}	10.8^{b}	9.3^{c}	12.5^{a}	9.6^{c}	11.2^{b}	10.4^{b}	12.0^{a}	0.19
OM (g/kg DM)	885	886	892	888	889	889	892	896	8.3
CP (g/kg DM)	218^{a}	199^{b}	203^{b}	185^{c}	193^{b}	185^{c}	208^{b}	191^{bc}	8.5
CHO (g/kg DM)	142^{d}	176^{c}	144^{d}	189^{b}	195^{ab}	206^{a}	156^{d}	203^{a}	9.6
NFC (g/kg DM)	453^{b}	495^{a}	470^{b}	507^{a}	482^{ab}	501^{a}	473^{b}	5.2^{a}	11.5
NDF (g/kg DM)	175	153	178	157	175	164	172	154	23.2
ADF (g/kg DM)	150	147	152	149	159	158	146	152	12.9
DOMD (g/kg DM)	813	831	835	855	836	849	828	841	14.5
ME (MJ/kg DM)	13.0	13.3	13.4	13.7	13.4	13.6	13.2	13.5	2.3
After vernalisation									
DM (g/kg DM)	124^{c}	14.2^{b}	13.3^{c}	14.9^{b}	16.5^{a}	17.6^{a}	16.3a	16.8^{a}	0.95
OM (g/kg DM)	859^{b}	867^{b}	868^{b}	868^{b}	890^{a}	896^{a}	895^{a}	901^{a}	5.8
CP (g/kg DM)	132^{a}	112^{b}	123^{a}	109^{b}	87.0^{c}	75.0^{c}	91.0^{b}	83.0^{c}	6.0
WSC (g/kg DM)	109^{b}	129^{a}	104^{b}	116^{ab}	111^{ab}	119^{ab}	94.0^{b}	105^{b}	7.1
NFC (g/kg DM)	571^{b}	601^{a}	553^{b}	606^{a}	454^{c}	477^{c}	417^{d}	456^{c}	11.5
NDF (g/kg DM)	268^{cd}	248^{d}	288^{c}	236^{d}	420^{b}	409^{b}	453^{a}	422^{b}	16.4
ADF (g/kg DM)	214^{b}	211^{b}	224^{b}	223^{b}	302^{a}	299^{a}	324^{a}	317^{a}	9.1
DOMD (g/kg DM)	718^{a}	736^{a}	712^{a}	730^{a}	611^{b}	634^{b}	582^{b}	617^{a}	10.2
ME (MJ/kg DM)	11.5^{a}	11.8^{a}	11.4^{a}	11.7^{a}	9.8^{b}	10.1^{b}	9.3^{b}	9.9^{b}	1.6

$^{a–c}$ Means within a row with different superscript letters differ significantly ($P < 0.05$).

DM – Dry matter; OM – Organic matter; CP – Crude protein; CHO – Soluble sugars and starch; NDF – Neutral Detergent Fibre; ADF – Acid Detergent Fibre; DOMD – Digestibility of the organic matter in the dry matter; ME – Metabolisable energy; NFC – Non fibre carbohydrates; NFC – [100- (NDF + CP + Fat + Ash)].

6.3.3 Dimensionality reduction analyses on herbage FA and nutrient components

Dimensionality reduction analyses failed to clearly identify differences in herbage FA and nutrient components between the two regrowth intervals before vernalisation. However, after vernalisation, there were clear differences between the two regrowth intervals on the aforementioned parameters, with minor overlaps in the ordination diagram depicted in Figure 6.1. The first two axis of the PCA were responsible for 82.2% of variation. The PCA showed differentiation of the nutrient components of the herbage into two clusters – associated with the two regrowth intervals. The longer regrowth interval was associated with higher concentrations of fibre (ADF, NDF) and OM, while the shorter interval was associated with greater concentrations of CP, NFC, ash, ME and DOMD. The individual and total FA in the herbage were in close proximity, in the same dimensionality reduction analysis quadrant and were clearly linked with the shorter regrowth interval.

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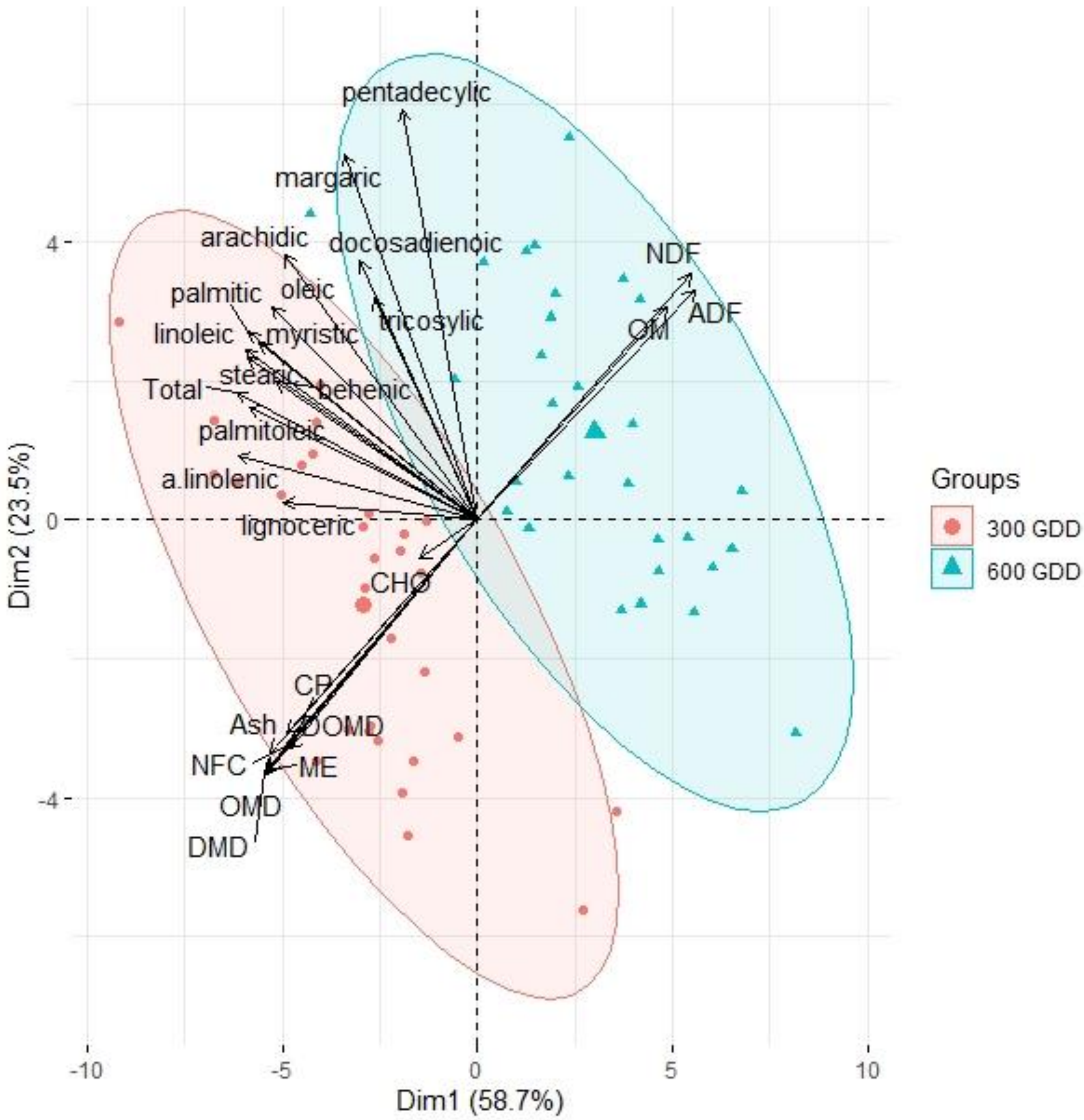

Figure 6.1. Bi-plot from Principal Component Analysis based on individual and total FA, as well as nutrient components of vernalised chicory herbage managed under two regrowth intervals (300 and 600 growing degree-days; GDD). Nutrient components abbreviation; CHO = soluble sugars and starch; CP = Crude protein; ME = Metabolisable energy; OM = organic matter; ADF and NDF = Acid and Neutral detergent fibre; DOMD; Dry organic matter digestibility; **NFC** = Non-fibre carbohydrates; Dim1 = Dimension 1; Dim2 = Dimension 2.

6.4 Discussion

The present study investigated whether regrowth interval, defoliation height and time of day influence the nutrient components and FA composition of chicory herbage before and after vernalisation. Prior to vernalisation, our results demonstrated little effect of management on plant chemistry, but after vernalisation, regrowth interval (300 GDD, 600 GDD) and, to a lesser extent, time of the day (morning, afternoon), influenced the FA profile and nutrient composition. Defoliation height did not have major impacts on the parameters measured either before or after vernalisation.

6.4.1 Fatty acid composition

6.4.1.1 Regrowth interval

This is the first study to evaluate the effects of contrasting regrowth intervals on the concentration of FA in chicory herbage before and after vernalisation. Our findings show that the effect of regrowth interval on herbage FA composition varied with the phenological stage of the plant. The relative lack of success of dimensionality reduction analyses to differentiate the FA composition of the herbage between the two regrowth intervals before vernalisation reflects the similarities in the morphology of the crop. Leaf proportion is vital in determining the overall FA levels in the herbage (Dewhurst et al., 2001). Our earlier study showed that all chicory plants were vegetative before vernalisation, with similar number of shoots and leaves across treatments (Mangwe et al., 2020c). Therefore, it is unsurprising that the herbage exhibited similar levels of FA, regardless of regrowth intervals before vernalisation.

The bi-plot generated a cluster of FA after vernalisation, with greater herbage FA concentrations associated with the shorter regrowth interval (Figure 1). Table 3 shows that extending regrowth interval from 300 GDD (29 calendar days) to 600 GDD (59 calendar days) after vernalisation reduced the concentration of LA, ALA and total FA by 28%, 40% and 33%, respectively. This result agrees with the patterns reported for other herbaceous plants in temperate regions. Dewhurst et al. (2001) found a decline of 33.5% and 56.6% in concentration of ALA for *Lolium perenne* and *Lolium multiflorum*, respectively, when the regrowth interval was extended from 20 days to 38 days. Elgersma et al. (2003a) noted a 14% and 19% decline in the concentration of total FA and ALA, respectively, for *Lolium perenne* when the regrowth interval was extended from 23 days to

33 days. The common basis for losses of FA in herbaceous plants with longer regrowth intervals in the previous studies was the increased proportion of stem material in the herbage. The FA concentrations in stem material of herbaceous plants was found to be 50% to 66% less than the concentrations found in leaves (Boufaïed et al., 2003). While this has not previously been reported in chicory, our findings showed 77% greater stem material in chicory herbage managed under the longer regrowth interval than those managed under the shorter regrowth interval. This may partially explain the decline in FA concentrations of herbage managed under longer regrowth intervals. Together with the results in the literature, our findings suggest that the leaf to stem ratio is vital in determining the concentration of herbage FA and highlights the importance of agronomic practices on FA composition of chicory herbage after vernalisation.

6.4.1.2 Diurnal changes

Browse et al. (1981) found an increase in the concentration of ALA and oleic acid from morning to evening in leaf blades of spinach (*Spinacia oleracea* L.) and maize (*Zea mays* L.). Similarly, Gregorini et al. (2008) noted an increase in the concentration oleic acid from morning to evening by 22 and 13% in cocksfoot (Dactylis glomerate L.) and meadow fescue (Festuca pratensis L.), respectively. Before vernalisation, there were no diurnal changes for all the FA measured in the current study, except for oleic acid. The lack of diurnal fluctuations in most of the predominant FA measured before vernalisation in the present study may be attributed to the maturity of the leaves during sampling. As a percentage of the biomass sampled, only a small part was developing leaves prior to vernalisation (Mangwe et al., 2020c), suggesting that most of the leaves were fully developed during sampling. It is well established that the FA metabolism and synthesis is greater in the developing leaves (Browse et al., 1981; Gregorini et al., 2008).

After vernalisation, there was some evidence for diurnal fluctuations on some individual FA in the herbage managed under the shorter regrowth interval. Of the predominant FA, diurnal fluctuations were observed for LA and oleic acid in the herbage managed under shorter regrowth intervals. However, no such trend was observed in the herbage managed under the longer regrowth interval. This may have been related to the increased proportion of stem material in the herbage managed under the longer interval, which might have diluted the concentration of FA in the herbage.

6.4.2 Nutrient composition

6.4.2.1 Regrowth interval

As with FA concentrations, there were no effects of regrowth intervals on the nutrient composition of the herbage before vernalisation. When comparing the effects of defoliating chicory every 250, 350 or 550 cm extended leaf height, Lee et al., (2015) also reported minor differences in nutrient composition (CP, fibre and digestibility) of chicory herbage in the first year before vernalisation. The nutrient components reported before vernalisation (CP > 185 g/kg DM; ME > 13.0 MJ/kg DM; NDF < 17.5 g/kg DM) are not expected to limit milk production (NRC, 2001). While the NDF content was less than the recommended 30-40% for lactating dairy cows diets to maintain optimum rumen functioning (NRC, 2001), chicory is rarely fed as sole diets in dairy cattle pastoral systems. Researchers have offered chicory with other forages to overcome the low NDF concentration encountered in pure chicory stands (Muir et al., 2015; Minneé et al., 2017; Mangwe et al., 2020b).

Regardless of regrowth intervals, the CP, ME and DOMD of the herbage declined, while the fibre content (ADF, NDF) increased after vernalisation. This pattern of change is consistent with the results of Belesky et al. (2000), Elgersma et al. (2014) and Lee et al. (2015) for chicory. Generally, plants in vegetative growth have a greater feeding value than their counterparts on reproductive growth stage as leaves have greater nutritive value than stems (Chen, et al. 2019; Ta et al., 2020). Our results further showed that longer regrowth exacerbated the decline in the feeding value of the herbage after vernalisation. Similarly, Elgersma & Søegaard (2017) noted that phenological stage of development and regrowth interval were the main determinants of the nutritive value of grass–legume mixtures during a two study conducted in a small-plot in Denmark. Several other studies investigating the impacts of defoliation frequency based on fixed number of days or extended leaf height on nutritive composition of chicory herbage have also reported similar patterns (Clark et al., 1990; Labreveux et al., 2006; Lee et al., 2015). The authors concluded that longer regrowth intervals after vernalisation decreased the feeding value due to the increased stem material in the herbage. In a review, Li and Kemp (2005) recommended that grazing management strategies should be designed to maintain a desirable 30:70 stem to leaf ratio. Our findings show that stem material accounted for 62% and 35% of total herbage biomass for longer and shorter regrowth intervals, respectively. Combined with the results in literature, the observations from this study confirm the important role of grazing management to control the growth and development of stems, and therefore, the nutrient composition of chicory herbage.

The CP requirement of dairy cows ranges between 140 to 180 g/kg of DM depending on stage of lactation (NRC, 2001). The low values reported in the current study (≤ 132 g/kg CP of DM) after vernalisation may limit milk production, especially 600 GDD herbage with 75 – 910 g/kg CP of DM. However, in another study, in which the CP concentration of the herbage ranged between 127 – 157 g/kg of DM, the low CP did not limit milk production (Mangwe et al., 2020b) because the cows selected the leaf material over the stem material. Allocation of chicory herbage rich in reproductive stems would need to consider stem refusal to avoid underfeeding and to achieve acceptable animal performance levels.

6.4.2.2 Diurnal changes

There were general declines in CP and fibre content as well as increases in CHO and NFC of the herbage from morning to afternoon across phenological stages. The magnitude of the diurnal changes in nutrient composition of non-vernalised chicory herbage reported in the present study is comparable to the study of Box et al. (2017). Smit & Elgersma (2004) found similar results, with strongest diurnal increases in CHO occurring in the top layer of the sward. Such increases in readily fermentable carbohydrates might synchronise the energy to protein balance, particularly with the high CP concentration present in the upper layer of the sward (Kebreab et al., 2001; Smit & Elgersma, 2004). Also, the decline in fibre content in afternoon herbage might reduce chewing effort, enhance forage particle size break down in the rumen and increase rumen passage rate, favouring increased herbage DM intake and animal production (Gregorini, 2012). In a study in which we evaluated the diurnal fluctuations in chemical composition of chicory on milk production, the results exhibited improvement in milk production from cows offered chicory in the afternoon when compared with the morning due to the diurnal changes in nutrient composition of chicory (Mangwe et al. 2020b). In a review, Edwards et al., (2007), reported that increases in readily fermentable carbohydrates to CP ratio improve N use efficiency, diminishing urinary N excretion and reducing the environmental impacts associated with pastoral livestock systems. Therefore, the higher fermentable carbohydrates to protein content ratio in afternoon herbage reported in the current study needs to be considered when designing grazing management strategies meant to curb the environmental challenges associated with livestock systems (Bryant et al., 2019).

6.4.3 Implications on milk FA concentration

Several reviews (Jenkins et al., 2008; Elgersma, 2015; Toral et al., 2018) postulated that the concentration of desirable PUFA in milk of ruminants could be influenced by the concentration of LA and ALA in the herbage. Increasing the concentration of PUFA in the diet of dairy cows can substantially improve both their concentration and that of CLA in ruminant-source foods (Elgersma et al., 2006). Muir et al., 2014; 2015) found 15 – 31 % higher concentration of PUFA in dairy cows fed chicory-based herbage compared with those fed PR. Similarly, our earlier findings exhibited enhanced milk PUFA concentrations from cows fed chicory-based diets compared with those fed PR (Mangwe et al. 2020a;b). The higher PUFA concentration in milk of animals grazing chicory-based herbage relative to PR was due at least in part to increases in PUFA concentration in chicory herbage (Muir et al., 2015; Mangwe et al., 2020). As a result, management practices that would enhance the concentration of PUFA in the herbage could impact the concentration of the desirable FA of milk. Our data suggest that, post vernalisation, shorter regrowth intervals will probably increase the levels of Omega-3 FA and CLA in milk of ruminants as compared to longer regrowth intervals due the increased concentration of their precursors in the herbage, although we are still dealing with quite small values.

Of the important FA, we observed diurnal changes in LA and oleic acid. While oleic acid is one of the nutraceutical FA in herbages, the low concentration of this FA in the herbage will render the diurnal changes insignificant. Linoleic acid, on the other hand, is one of the major precursors of the desirable FA in ruminants products (Lock & Bauman, 2004). The increases in CLA concentration in milk of cows on fresh pasture when compared with those on concentrates or conserved forages has been found to be related to the greater concentrations of LA and ALA in the fresh herbage (Elgersma et al., 2003b; Dewhurst et al., 2006). Further studies that will examine the potential beneficial impacts of the greater concentration of LA in afternoon on the CLA concentration in the milk of animals are strongly desirable.

6.5 Conclusion

The study demonstrated a significant regrowth interval effect on the FA composition and chemical composition of chicory herbage after the plants were vernalised, suggesting a potential for pastoral farmers to manipulate grazing management practices to enhance FA content and feeding value of the forage. Overall, the feeding value and the concentration of FA in the herbage were greater on chicory herbage managed under shorter regrowth intervals than that under longer regrowth intervals, which is likely to favour animal production and the concentration of the desirable FA in ruminant products. The concentration of LA and oleic acid in the herbage increased during the day in plants under shorter intervals after vernalisation, however, ALA and total FA concentrations remained stable throughout the day. Further studies are required to determine if the increase in LA in afternoon herbage will enhance the concentration of desirable FA in ruminant products.

Chapter 7

7. Rumen fermentation and fatty acid composition of milk of mid lactating dairy cows grazing chicory and ryegrass

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Conceptualization, review, editing and co-author of manuscript.

The content of this Chapter is exact copy of the journal paper referred to above. The original paper has been formatted for consistence within the thesis.

7.1 Introduction

Enhancing the concentration of individual fatty acids (FA) in milk has been, for a long time, an important aim for researchers because certain FAs are linked with health benefits in humans (Chilliard et al., 2000b) as well as improving the processing quality of milk products. Fifty percent of the FA found in milk are sourced from the blood and the remaining 50% are synthesized in the mammary gland (National Research Council Committee, 1988). Those FA synthesized in the mammary gland tend to be short-chain acids (C4:0 to C14:0) and are largely influenced by animal genetics (Knutsen et al., 2018). The FA sourced from blood are predominantly of diet or microbial origin, with lipolysis and the mobilization of body fat accounting for 5% in a well-fed animal to over 20% of milk FA in early lacttion when cows are in a negative energy balance (Bauman and Griinari, 2003; Lock and Bauman, 2004). The content and composition of FA of microbial origin varies markedly, and typically represent the odd-chain and branched FAs (OBCFA). Researchers have attempted to use these milk FAs to predict volatile fatty acid (VFA) production in the rumen as a measure of diet effect on rumen function (Vlaeminck et al., 2006).

Milk FA derived from the diet is also variable and represents long chain polyunsaturated fatty acids (PUFA), which have been linked to several positive human-health related effects (Palmquist, 2009). Diet FA, particularly PUFA, are extensively biohydrogenated in the rumen, which reduces their concentration in milk (Jenkins et al., 2008). Plant factors can influence this process, providing opportunities to manipulate FA proportions in the rumen and thereby in the milk (Toral et al., 2018). For example, diets high in readily fermentable carbohydrates are known to increase total VFA and reduce pH during ruminal fermentation, limiting lipolysis, and thus, biohydrogenation (Chouinard et al., 1999; Kolver and De Veth, 2002). Chilliard et al. (2000b) reported a 35% to 50% decrease in ruminal biohydrogenation of PUFA, such as linoleic (LA; C18:2 c9, 12) and α-linolenic acid (ALA; C18:3 c9, 12, 15) when concentrates formed more than 70% of the diet, a result of reduced biohydrogenation at lower rumen pH.

Alternative forages, such as chicory (CH; *Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.) present an opportunity to improve the FA composition of milk whilst meeting environmental and economic requirements in pastoral livestock systems. Chicory has long been considered a useful component of the pastoral system in temperate regions (Li and Kemp, 2005), providing benefits of improved mineral nutrition (Crush and Evans, 1990; Barry, 1998a) and producing a large amount of high-quality summer feed compared with RGWC when sown as monoculture or in diverse swards (Glassey et al., 2013; Minneé et al., 2013; Nobilly et al., 2013). Chicory contains more readily fermentable carbohydrates (non-fibre carbohydrates; NFC) than ryegrass/white clover (*Lolium perenne* L./*Trifolium repens* L.; RGWC) herbage (Minneé et al., 2017), which may increase the concentration of total VFA and lower rumen pH during ruminal fermentation. In our earlier proof-of-concept research, elevated PUFA were recorded from late lactation cows fed pasture diets of CH compared with the control RGWC in a grazing study (Mangwe et al., 2018). Feeding CH, as all or as part of a ration, has also shown milk yield improvements (Chapman et al., 2008; Mangwe et al., 2019) and nitrogen loss reductions (Totty et al., 2013; Bryant et al., 2017; Minneé et al., 2017) compared with traditional ryegrass pastures.

However, Chapman et al. (Chapman et al., 2008) pointed out the need to consider strategies to integrate alternative feeds into farm systems. Although our previous research showed that at 100% of the diet, CH could improve milk FA profile and milk production, it is not feasible to include CH at 100% of pastoral diets for extended periods. Muir et al. (Muir et al., 2014), feeding partial mixed ration demonstrated the feasibility of feeding CH at 50% and increasing milk PUFA. To capture the value of alternative forages as a means for improving product quality in terms of milk FA, more information is required to understand the mechanisms leading to increased PUFA from CH diets and associated feeding management. In a review, Gregorini (2012) showed that a small change from the routine allocation of fresh herbage of one forage species could have positive benefits on animal performance and environmental impact. Indeed, Abrahamse et al. (2009) demonstrated that allocating pasture to dairy cows in the afternoon compared with the morning altered milk composition and fat yield.

To better understand the rumen fermentation factors influencing milk FA profile of CH herbage and identify suitable feeding regimes to capture forage derived benefits on milk quality, a grazing study was conducted comparing rumen fermentation and fatty acid composition of rumen and milk of mid-lactating dairy cows on CH or conventional RGWC pastures.

7.2 Materials and methods

7.2.1 Experimental site and design

The experiment took place between 10 December 2018 and 27 January 2019 at the Lincoln University Research Dairy Farm, about 20 km south of Christchurch in Canterbury, New Zealand (43°38′S, 172°28′E; 17 m above sea level) with the approval of the Lincoln University Animal Ethics Committee (AEC #2018-48). The experiment was organized in a completely randomized design with three replicated feeding regimes: (1) perennial ryegrass/white clover only (RGWC), (2) ryegrass/white clover + morning allocation of chicory (CHAM) and (3) ryegrass/white clover + afternoon allocation of chicory (CHPM).

The pastures used in this experiment were second-year CH and sixth-year RGWC. Details of establishment were given in Mangwe et al. (2019). Briefly, the ryegrass (cv. Arrow AR1; 20 kg/ha) and white clover (cv. Weka; 3 kg/ha) swards were established in October 2013 while CH (cv. Choice, 5.3 kg seed/ha) swards were established in November 2017 following cultivation. The soil was classified as free-draining Templeton fine sandy loam soil (Hewitt 2010) with a soil pH of 6.2 (1: 2.1 *v/v* soil–water slurry), Olsen phosphorus of 29.7 mg/L, potassium of 0.9, calcium of 8.2, magnesium of 1.1, and sodium of 0.2 me/100 g as determined on 29 September 2017 to 75 mm depth. We did not apply any fertilizer during establishment. For the current research, the experimental area of 10.5 ha consisting of 7 × 1.5 ha paddocks was prepared three to four weeks prior to the study. To ensure that all plants had accumulated similar growing degree days during the experiment and to build a feed wedge, a third of each paddock was rotationally grazed using a group of cows, and mowing after grazing to a uniform height of 4 cm. Nitrogen fertilizer was applied at 30 kg N/ha as urea immediately after grazing each paddock.

7.2.2 Animals and management

The experiment included a 4-week baseline measurement period, where all cows grazed RGWC plus 15–20% of the diet as CH herbage daily, a 6-day adaptation period in which the relative proportion of the diet was increased to 50% of the diet, and a 12-day measurement period. Based on results obtained during the baseline measurement period, 36 mid-lactating Friesian × Jersey dairy cows on their second to fourth parities were stratified into nine groups of four cows and assigned to one of the three replicated (n = 3) feeding regimes (RGWC, CHAM, and CHPM). One cow per group had a rumen cannulae fitted (Bar-Diamond; Parma, Idaho, USA). Cows were stratified according to (mean ± standard error of the mean (SEM)); milk fat content (5.08 ± 0.25 g/100 g of milk), milk protein content (3.78 ± 0.06 g/100 g of milk), milk solid yield (MS; 1.82 ± 0.08 kg/cow per d), milk yield (21.3 ± 0.97 kg/cow per d), days in milk (155 ± 3.3 days), and live body weight (483 ± 13.8 kg).

Both CH and RGWC herbages were grazed in situ using similar herbage allowance. Target allowance was 34 kg of dry matter (DM) per cow per day above ground level to maintain baseline milk production. Allocations were based on herbage mass determined every three days by harvesting to ground level herbage within three 0.25-m^2 quadrat cuts per break, and weighing the washed, dried material. Details of the management regimes during the experiment are summarized in Figure 7.1. Briefly, control cows offered RGWC received a fresh allocation (34 kg DM/cow per day above ground) after 24 hours, following the morning milking. Cows offered either of the CH treatments were allowed to graze CH for five and a half hours before returning to RGWC. The cows on CHAM received a new allocation of CH herbage (17 kg DM/cow per day) between morning and afternoon milking (0800 and 1330 h) and a new allocation of RGWC herbage (17 kg DM/cow per day) following afternoon milking. Cows offered CHPM received a new allocation of RGWC herbage (17 kg DM/cow per day) between morning and afternoon milking, a new allocation of CH herbage (17 kg DM/cow day) following afternoon milking (1600–2130 h), after which they went back to their previous RGWC allocation. Temporary fencing was used to control cows. All cows had free access to fresh water at all times.

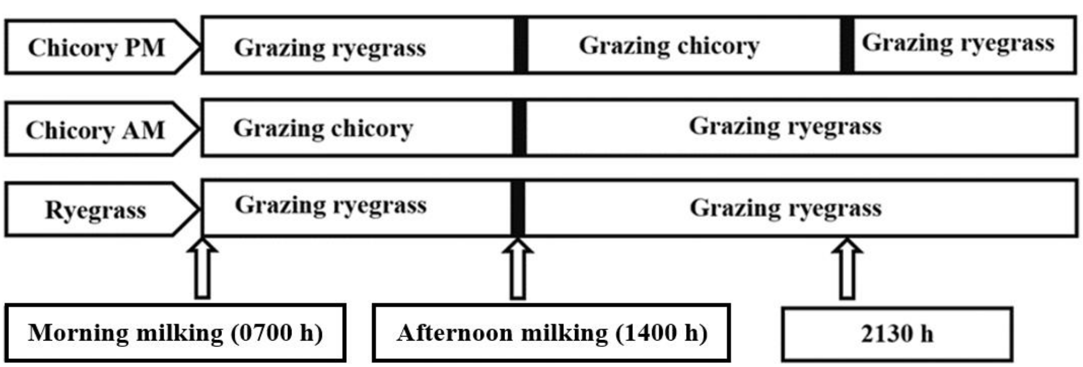

Figure 7.1**.** Management regimes during the experiment. Control cows offered ryegrass only (RGWC), received a fresh allocation after 24 hours following the morning milking (around 0800 h). Cows on Chicory AM received a new allocation of chicory herbage between morning and

afternoon milking (0800 h and 1330 h) and a new allocation of ryegrass herbage following afternoon milking. Cows offered Chicory PM received a new allocation of ryegrass herbage between morning and afternoon milking, a new allocation of chicory herbage following afternoon milking (1530 h–2100 h), after which they went back to their previous ryegrass allocation.

7.2.3 Herbage measurements

Representative herbage samples for chemical and botanical composition were harvested to ground level from each of the mornings and the afternoons' allocations preceding the cows moving into their allocations (0700 h and 1300 h, respectively) on day 6, 8, 11, 16, and 18 of the experiment. Samples were transported to the research facilities, homogenized, and sub-sampled for botanical and chemical analysis. Each sub-sample was separated into sown species, reproductive, vegetative, weed, and dead material. The separated components were dried at 60 °C for at least 48 hours. The DM content of the homogenized sub-sample was determined by immediately recording fresh weight and dry weight after oven drying at 60 °C for 48 hours. The remaining homogenized fresh herbage sample was freeze-dried and ground to pass a 1-mm sieve (ZM200 Retsch). Organic matter (OM), water-soluble carbohydrates (WSC), neutral and acid detergent fibre (NDF, ADF), crude protein (CP), dry matter digestibility (DMD), organic matter digestibility (OMD), and digestible organic matter in the dry matter (DOMD) from the dried ground samples were estimated using near-infrared spectrophotometry (NIRS, Model: FOSS NIRSystems 5000, Maryland, USA). The NIRS calibration for WSC (MAFF, 1986), crude protein (Variomax CN Analyser, Elementar), NDF (Van Soest et al., 1991) and ADF (method 973.18; AOAC, 2012), DOMD, and DMD (Iowerth et al., 1975) were previously derived from RGWC and CH forages. All R-squares for predicting the nutrients measured were similar and were above 0.9. All samples were well within the calibration range.

Total dry matter intake (DMI) was estimated on day 6, 9, 11, 16, and 18 of the experiment as the sum of CH and RGWC herbage apparent intakes. Apparent intake was estimated using the formula; Intake (kg DM/cow/day) = (((pre kg DM ha^{-1} – post kg DM ha^{-1}) ÷ No. cows) × area). Pre-graze and post-graze were based on herbage harvested to ground level within three 0.25-m^2 quadrats before and after grazing respectively.

7.2.4 Milk yield and composition measurements

Milk yield was recorded daily at 0700 and 1400 h with an automated system (DeLaval Alpro Herd Management System, DeLaval, Tumba, Sweden). Individual cows' milk was sampled on days 6, 8, 11, 16, and 18 of the experiment for further analysis. Milk fat, protein, and lactose contents were determined from fresh milk using Milkoscan™ (Foss Electric, Hilleroed, Denmark). Milk samples for FA composition were taken from individual cows on day 16 and 18 of the experiment.

7.2.5 Grazing behaviour

Three cows in each group were fitted with SensOor ear-tags (Agis Ltd, the Netherlands) to continuously record time spent grazing, ruminating and idling per day.

7.2.6 Rumen sampling

Rumen fluid and digesta samples were collected from the nine ruminal cannulated cows at four-hourly intervals starting from 0400 to 2400 h on day 13 and 15 of the experiment. Rumen digesta samples were collected by hand, via the rumen cannulae, from the mid and dorsal rumen where fermentation is most active. Rumen fluid samples for determination of VFA were attained by squeezing a subsample of the composited rumen digesta through two layers of cheesecloth and stored at -20 °C pending analysis. Volatile fatty acids concentration from rumen fluid samples were determined using a Gas Chromatograph (GC: Shimadzu GC-2010, Kyoto, Japan) fitted with an SGE BP21 30 m × 530 µm × 1.0 µm wide-bore capillary column. The remaining rumen digesta sample was mixed, subsampled, placed in resealable plastic bags and immediately stored at −20 °C pending FA analysis. Each of the nine cows had intra-ruminal smaXtec pH sensors (Smart Farm Data Limited, New Zealand) inserted before the experiment to continuously measure pH, but software failure resulted in an incomplete data set. Ruminal pH was therefore measured from thawed rumen fluid samples taken at four-hourly intervals, using pH probe (HD 2105.2 pH/mv meter; Delta Ohm Inc., Padua, Italy). Samples were kept chilled during pH measurements.

Rumen digesta, herbage and individual cow milk samples for FA acid were prepared by transmethylation and analyzed by gas chromatography (with AOC-20i auto-sampler, Shimadzu GC-2010, Japan), according to AOAC (2012) Method 2012.13 using a Varian CP742 silica capillary column (0.25 × 100 m × 0.2 µm).

7.2.7 Statistics and calculations

For all analyses, we used a mixed-effects model in R. The animal group (paddock) was used as an experimental unit. For data taken from all cows (milk yield, composition, and FA profile), treatment (CHAM, CHPM, and RGWC) was included as a fixed effect, while animal nested in sampling day used as random effect. For data taken at paddock level (herbage composition and intake), treatment and forage type (CH and RGWC) and their interaction were included as fixed terms while day used as random effect. To explore diurnal patterns in rumen fermentation parameters and rumen FA composition, treatment was included as fixed effect, sampling time (0400, 0800, 1200, 1600, 2000, 2400 h) as a repeated measure, while animal nested on sampling day used as random effect. For all data, means separation was done using the 'emmeans' package of R, with Tukey's method for comparing the estimates. A significant difference was declared at $P < 0.05$, while a tendency was declared at $P < 0.10$.

7.3 Results

7.3.1 Herbage characteristics

In CH pastures, mean CH herbage accounted for an average of 821 ± 21 g/kg of the biomass, while ryegrass accounted for 612 ± 32 g/kg on a dry weight basis in RGWC pastures. Chicory swards were at reproductive stage, with the reproductive stem accounting for an average of 394 ± 14 g/kg DM of the CH herbage. The reproductive stem in ryegrass herbage accounted for 121 ± 5.9 g/kg of DM. Chicory swards had less than 50 g/kg white clover, while ryegrass swards had 93 ± 8.7 g/kg of DM white clover. In RGWC swards, dead material accounted for 192 ± 11 g/kg and weed content accounted for 114 ± 7.8 g/kg of DM. The corresponding proportions of dead material and weed content were less than 50 and 101 ± 6.6 g/kg of DM, respectively, on CH pastures.

Herbage mass and pre-grazing chemical composition are presented in Table 7.1. Treatment did not affect pre- and post-graze mass. However, CH herbage was grazed to a lower residual height than RGWC herbage (1387 kg/ha DM *vs.* 1700 kg/ha DM; $P < 0.001$). Water-soluble carbohydrates, ADF, crude fat and digestibility were similar for all treatments, whereas CP, NDF, and NFC differed between treatments. There was an interaction between herbage type and time of allocation for DM, NDF, DOMD, and NFC ($P < 0.05$). Generally, CH herbage had greater NFC, but less

DM, OM, CP, NDF, and ADF contents than RGWC ($P < 0.05$). Herbage offered in the afternoon had greater concentrations of DM, NFC, and DOMD than herbage allocated in the morning, regardless of herbage type.

Time of allocation did not affect total diet FA (Table 7.1; $P > 0.05$), but CH herbage had a greater concentration of total FA than RGWC herbage (24.3 vs. 18.4 ± 1.89 mg/g DM). The predominant FAs in the herbage were LA and ALA, which accounted for 27.1 and 47.9%, respectively in CH and 16.9 and 57.6% in RGWC herbage, respectively.

The reproductive stem in the herbage after grazing doubled, highlighting selection against these plant components by cows as stem accounted for an average of 703 ± 24 g/kg DM in CH swards and 225 ± 6.2 g/kg DM in RGWC swards. Post grazed CH herbage had 389, 103, and 635 g/kg, NDF, CP, and DOMD, while RGWC herbage had 559, 110, and 634 g/kg, NDF, CP, and DOMD, respectively.

Table 7.1. Herbage mass, pre-grazing chemical composition and fatty acid (FA) profile of chicory and ryegrass/white clover (RGWC) sampled to ground level.

Herbage	Chicory herbage		Ryegrass/white clover herbage			SEM[5]	P - value		
Treatments	**CHAM[1]**	**CHPM[1]**	**CHAM[1]**	**CHPM[1]**	**RGWC[1]**		**Treatment**	**Herbage**	**T x H**
Pre-graze mass (kg/ha DM)	2985	3150	2958	2960	3277	175	0.375	0.541	0.645
Post-graze mass (kg/ha DM)	1372	1402	1670	1740	1687	83	0.282	< 0.001	0.812
Organic matter (g/kg DM)	868	881	922	921	918	5.1	< 0.001	< 0.001	0.152
Dry matter (g/kg DM)	119	135	220	194	212	5.4	< 0.001	< 0.001	0.004
Water soluble carbohydrates (g/kg DM)	145	197	233	210	206	7.8	0.196	0.011	0.075
Crude protein (g/kg DM)	146	127	157	153	165	6.4	0.024	0.014	0.293
Neutral detergent fibre (g/kg DM)	296	246	446	473	455	9.4	< 0.001	< 0.001	< 0.001
Acid detergent fibre (g/kg DM)	230	214	263	268	262	4.2	0.1	< 0.001	0.191
Dry matter digestibility (g/kg DM)	782	798	743	736	748	5.2	0.373	< 0.001	0.281
DOMD[2] (g/kg DM)	710	757	737	706	729	10.4	0.579	0.112	< 0.001
Crude fat (g/kg DM)	38.8	41.8	41.8	45.1	42.6	0.8	0.248	0.099	0.942
NFC[3] (g/kg DM)	401	454	277	250	256	11.5	< 0.001	< 0.001	0.002
Fatty acids (mg/g DM)									
C14:0	0.06	0.062	0.10	0.09	0.08	0.01	0.501	< 0.001	0.503
C16:0	4.76	4.62	3.51	3.35	3.14	0.3	0.034	< 0.001	0.968
C18:0	0.23	0.23	0.32	0.31	0.29	0.02	0.562	< 0.001	0.692
C18:1 c9	0.39	0.39	0.39	0.38	0.41	0.03	0.844	0.902	0.921
C18:2 c9,12	6.71	6.48	3.23	3.13	2.99	0.38	< 0.001	< 0.001	0.864
C18:3 c9,12,15	11.1	12.2	10.9	10.8	10.1	1.25	0.438	0.295	0.395
Saturated FA	5.49	5.34	4.33	4.14	3.91	0.33	0.056	0.002	0.968
Monounsaturated FA	0.53	0.53	0.61	0.58	0.58	0.46	0.86	0.20	0.81
Polyunsaturated FA	18.8	17.7	14	14.1	13.1	1.56	0.137	0.005	0.471
Others[4]	0.65	0.62	0.66	0.64	0.610	0.03	0.530	0.705	0.869
Total FA	24.9	23.6	19	18.8	17.5	1.89	0.122	0.005	0.561

[1]RGWC = perennial ryegrass/white clover only; CHAM = ryegrass/white clover + morning allocation of chicory; CHPM = ryegrass/white clover + afternoon allocation of chicory.

[2]DOMD = Digestibility of the organic matter in the dry matter

[3]NFC = Non-fibre carbohydrates (1000- (NDF + CP + Fat +Ash)).

[4]Others = C15:0; C16:1 c9; C17:0; C18:1 c11; C20:0; C20:2 c11,14; C20:3 c11,14,17; C22:0; C23:0; C24:0

[5]SEM = standard error of the mean.

7.3.2 Dry matter intake and grazing behaviour

Feeding regime did not affect apparent DMI (Table 7.2). On average, CH accounted for 55% and 58% of total DMI for cows offered the CHAM and CHPM, respectively. Total time spent grazing per day was also unaffected by feeding regime (499 ± 15 min/cow per day; P = 0.167), but cows offered RGWC spent more time ruminating (446 ± 9.34 min/cow per day) than those offered CHAM (379 ± 9.69 min/cow per day) or CHPM (359 ± 10.13 min/cow per day; P <.0001). Irrespective of treatment, cows consumed the majority of forage during two major grazing bouts (0900–1300 h and 1600–2000 h; Figure 7.2). When cows were grazing CH, the intensity (minutes/h) and duration of their grazing was greater than on RGWC. Cows offered CHAM grazed more intensely in the morning, spending nearly 211 vs. 109 and 150 minutes/5 h between 0900–1300 h compared with CHPM and RGWC, respectively. Whereas cows offered CHPM grazed more intensely during the afternoon, spending 212 minutes vs. 140 and 167 between 1600–2000 h compared with CHAM and RGWC, respectively.

Table 7.2. Estimated dry matter intake (DMI; kg/cow per day of DM), milk yield and milk composition from cows fed grazing CHAM[1], CHPM[1], and RGWC[1].

Item	CHAM[1]	CHPM[1]	RGWC[1]	SEM[2]	P-value
Chicory intake	9.00	9.31	-	0.57	0.578
Ryegrass intake	7.34b	6.81b	16.6a	0.23	<0.0001
Total DMI	16.3	16.1	16.6	0.25	0.122
Milk yield (kg/cow per d)	21.0ab	22.0a	19.9b	0.43	<0.0001
Milk solids (kg/d)	1.84b	1.96a	1.71c	0.03	<.0001
Fat (g/100 g of milk)	4.95	5.14	4.92	0.10	0.051
Protein (g/100 g of milk)	3.85a	3.75b	3.71b	0.03	0.002
Lactose (g/100 g of milk)	5.06	5.05	5.07	0.02	0.662
Protein: Fat	0.79a	0.74b	0.76b	0.01	0.004
Fat yield (kg/d)	1.04b	1.13a	0.97b	0.02	<0.0001
Protein yield (kg/d)	0.82a	0.83a	0.74b	0.02	<0.0001
Lactose yield (kg/d)	1.06ab	1.11a	1.01b	0.02	<0.0001

[a-c] Means within a row with different letters differ (P < 0.05).[1] RGWC = perennial ryegrass/white clover only; [1]CHAM = ryegrass/white clover + morning allocation of chicory; CHPM = ryegrass/white clover + afternoon allocation of chicory.[2] SEM = standard error of the mean.

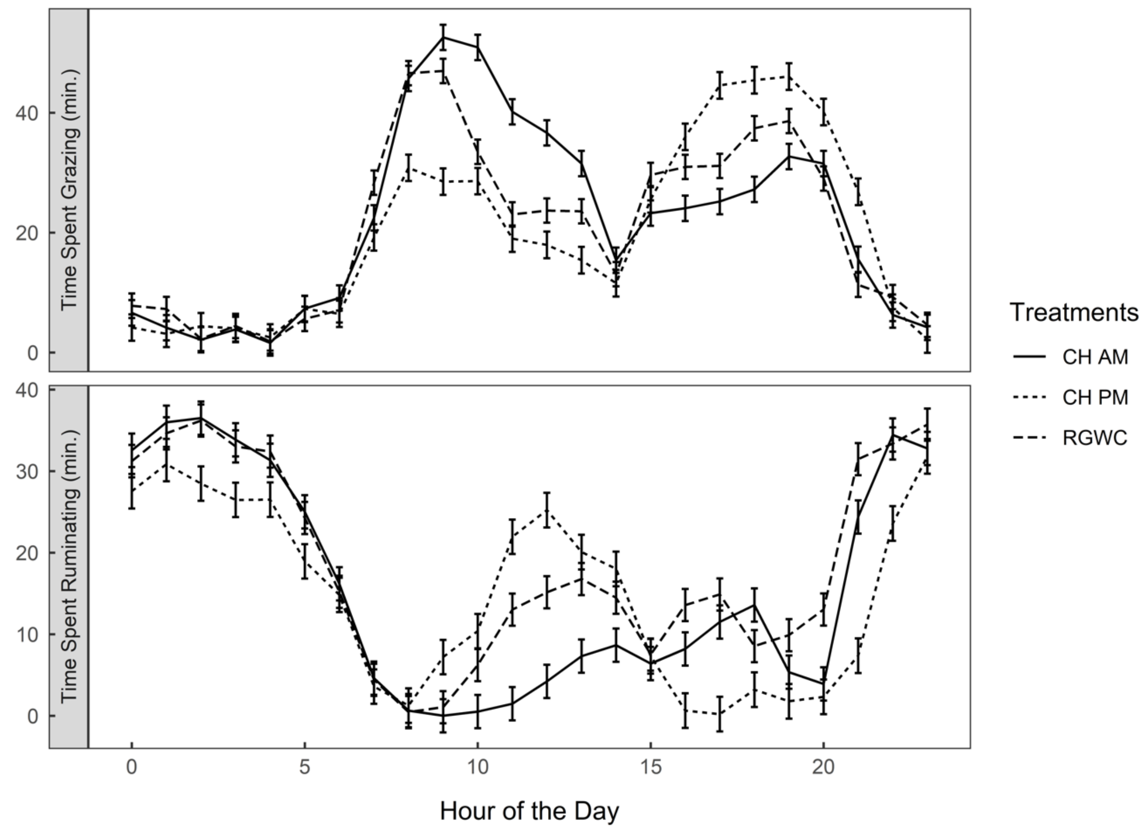

Figure 7.2**.** Diurnal variation of time spent grazing and time spent ruminating (minutes/hour). Solid lines denote CHAM (ryegrass/white clover + morning allocation of chicory), short dashed line denotes CHPM (ryegrass/white clover + afternoon allocation of chicory), and long dashed line denote RGWC (perennial ryegrass/white clover only). Shaded areas represent AM and PM milking events. Error bars are standard error of the mean. Abbreviation; CH, chicory.

7.3.3 Milk production and composition

When compared with RGWC, including CH increased milk production (Table 7.2). Although CHPM and CHAM had similar milk yield, elevated milk fat percent (P = 0.051) and milk fat yield, for CHPM resulted in greater milk solids than CHAM. Milk protein percent was increased in cows offered the CHAM compared with those offered the CHPM or RGWC.

Cows on CHAM and CHPM feeding regimes had milk with similar concentrations of LA and ALA, but greater than the RGWC (Table 7.3). Cows on RGWC increased vaccenic acid (VA; C18:1 t11) compared with the CHAM or CHPM regimes. Conjugated linoleic acid (c9, t11 C18:2; CLA) was similar for cows on CHAM and RGWC but tended to be greater for those on RGWC than those on CHPM (P = 0.05). Odd and branched-chain FA such as isoC15:0, anteisoC15:0, C17:0, and isoC17:0 were greater in cows fed RGWC than those fed CH. The sum of saturated fatty acids was not affected by feeding regime (P = 0.60), but CH inclusion increased the concentration of PUFA in the milk, regardless of the time of allocation (P < 0.0001).

Table 7.3**.** Milk fatty acid (FA) composition (g/100 g of FA) from cows fed CHAM [1], CHPM [1], and RGWC[1].

Item	CHAM	CHPM	RGWC	SEM[4]	P-value
C4:0:C12:0	17.2	15.8	15.5	0.44	0.406
C14:0	11.5	11.1	11.0	0.3	0.131
C14:0iso	0.09b	0.10b	0.11a	0.004	0.003
C15:0iso	0.28b	0.27b	0.32a	0.007	<0.0001
C15:0	1.19	1.15	1.17	0.025	0.368
C15:0anteiso	0.56	0.56	0.62	0.053	0.589
C16:0	31.9	32.8	32.8	0.67	0.369
C16:0iso	0.23	0.23	0.24	0.007	0.512
C17:0	0.55b	0.55b	0.59a	0.026	0.02
C17:0iso	0.43b	0.44b	0.49a	0.036	0.008
C17:0anteiso	0.56	0.55	0.54	0.014	0.611
C18:0	11.2	11.7	12.3	0.5	0.723
C18:1t11	2.49b	2.43b	3.02a	0.333	0.007
C18.1c9	16.4	17.1	16.7	0.563	0.553
C18:2 c9,12	1.16a	1.21a	0.90b	0.059	<0.0001
C18:3 c9,12,15	1.12a	1.18a	0.94b	0.04	<0.0001
c9 t11 CLA	0.94	0.87	1.10	0.068	0.059
C20:0	0.12	0.11	0.26	0.005	0.0007
C22:0	0.08	0.08	0.08	0.008	0.945
C20:5 c5,8,11,14,17	0.11a	0.10b	0.10b	0.003	0.033
C22:5 c7,10,13,16,19	0.12	0.12	0.11	0.004	0.619
Saturated FA	73.4	72.2	72.4	0.581	0.954
Monounsaturated FA	22.2	23.5	23.7	0.588	0.6
Polyunsaturated FA	4.28a	4.38a	3.9b	0.074	<0.0001
de novo[2]	25.9	24.9	24.7	0.69	0.051
Omega-3	1.35a	1.39a	1.15b	0.042	<0.0001
Omega-6	1.16a	1.21a	0.90b	0.059	<0.0001
Trans	3.74ab	3.51b	4.11a	0.16	0.025
Others[3]	6.01	6.2	5.3	0.89	0.114

[a–b] Means within a row with different letters differ (P < 0.05). [1] RGWC = perennial ryegrass/white clover only; CHAM = ryegrass/white clover + morning allocation of chicory; CHPM = ryegrass/white clover + afternoon allocation of chicory.[2] *de novo* includes fatty acids with <16 carbon atoms.[3] Others include C14:1c9, C16.1t9, C16.1c7, C16.1c9, C18.1c6, C18.1c11, C18.1c12, C18.1c15, C18.1t9, C18.1t10, C18.2t9c12, C18.2c9t13, C20.1c8.[4] SEM = Standard error of the mean.

7.3.4 Rumen fermentation parameters

Major VFAs (acetic, propionic and butyric acid) accounted for nearly 97% of total VFA. The four-hourly diurnal variations in the individual VFAs demonstrate the time × treatment interaction and reflect the variation in feeding patterns of the different regimes (Figure 7.3). However, on average cows offered CH (CHAM and CHPM) had greater total concentrations of VFA than animals grazing RGWC (140 *vs.* 128 ± 4.3 mmol/L; $P < .0001$).

Fluctuations in VFA profiles corresponded with changes in rumen pH (Figure 7.3). For all feeding regimes, ruminal pH was highest (pH = 7.1 ± 0.17) at the end of the allocation period between 0400 and 0800 h. Rumen samples taken at 1200 h, four hours after the morning allocation of fresh herbage, indicated significant reductions in pH of all treatments. However, pH was more reduced for cows on CHAM (pH = 5.72 ± 0.15), intermediate for cows on CHPM (pH = 6.11 ± 0.17) and least affected for cows on RGWC (pH = 6.53 ± 0.15). The complete VFA concentration in ruminal contents in cannulated cows is presented in S2 Table.

7.3.5 Rumen long-chain FA composition

The mean concentration of LA in the rumen was 6.99, 7.71, and 6.08 ± 0.45 g/100 g of total FA, while that of ALA was 7.61, 7.76, and 6.11 ± 1.1 g/100 g of total FA for cows on CHAM, CHPM, and RGWC feeding regimes, respectively. Diurnal patterns of selected rumen FA are depicted in Figure 7.4. There was a significant feeding regime × sampling time interaction for LA, ALA, VA, and stearic acid (C18:0). Rumen LA and ALA concentrations were 34% and 56% greater for cows on CHAM than cows on RGWC or CHPM at 1200 h. Cows on CHPM had 90% greater rumen ALA at 2000 h compared with cows on RGWC or CHAM. The increase in these plants derived PUFA corresponded with a sharp decline in biohydrogenation intermediate VA and biohydrogenation end-product stearic acid.

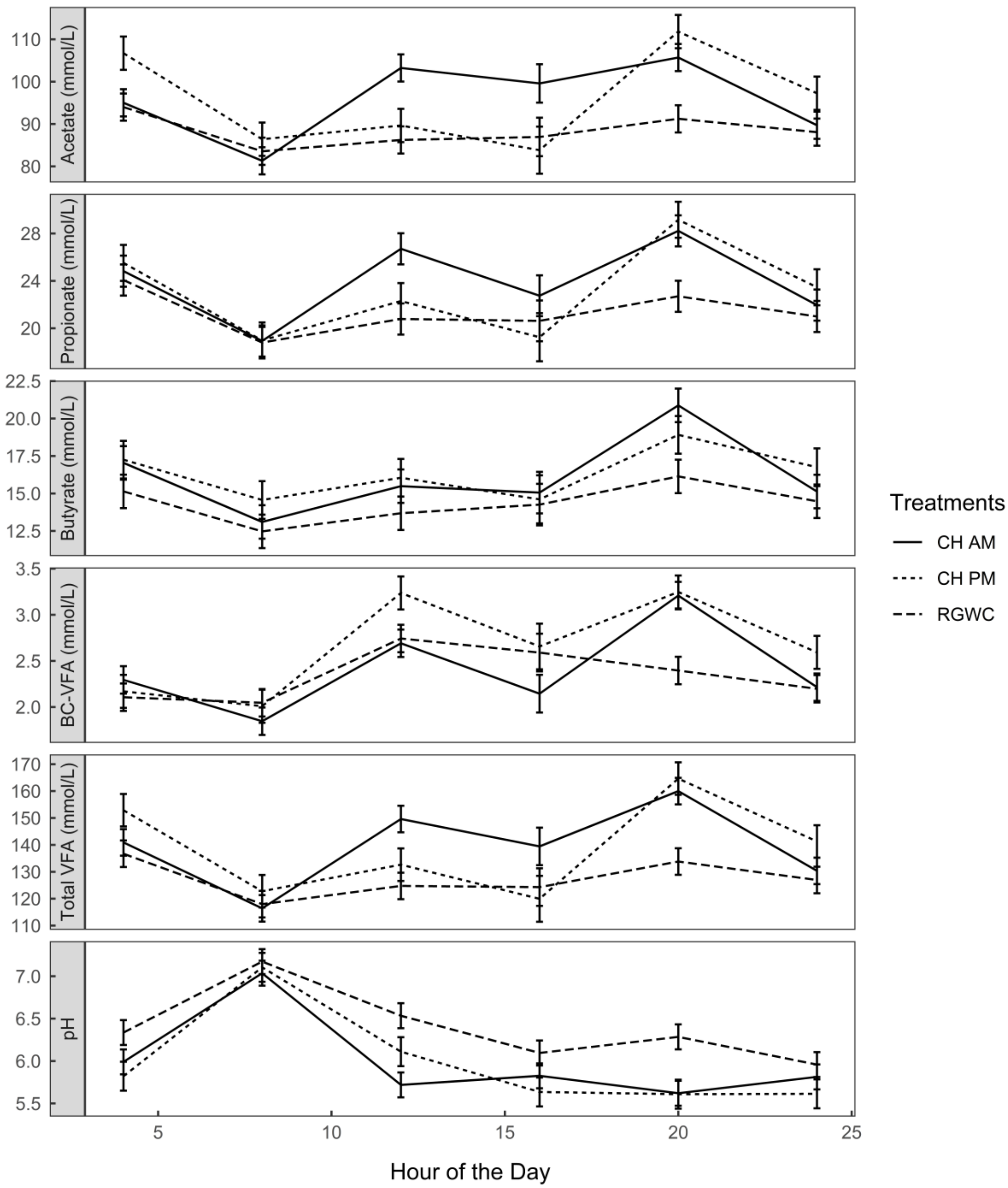

Figure 7.3**.** Diurnal variation of rumen fermentation parameters. Solid lines denote CHAM (ryegrass/white clover + morning allocation of chicory), short dashed line denotes CHPM (ryegrass/white clover + afternoon allocation of chicory), and long dashed line denote RGWC (perennial ryegrass/white clover only). BC-VFA (branched chain-volatile fatty acids; iso-valerate + iso-butyrate). Error bars are standard error of the mean (n = 3).

7.4 Discussion

This is the second study in a series of experiments investigating the effect of high moisture forages on the production and quality of milk and associated environmental impacts in pastoral dairy systems. Our first proof-of-concept study showed that under pastoral grazing feeding high moisture, herb diets altered milk FA composition without affecting production (Mangwe et al., 2018). The results of the present study confirm the positive effect of feeding CH on PUFA and demonstrate that producers can influence FA composition through changes in feeding regimes.

7.4.1 Milk production and rumen fermentation

It is interesting that feeding reproductive CH at 55–58% of the diet of mid lactating dairy cows increased milk production compared with the control-feeding regime, a result similar to previous findings when CH was fed while vegetative (Chapman et al., 2008; Mangwe et al., 2018). Muir et al. (2015), on the other hand, did not observe any differences in milk production between the control ryegrass and reproductive CH at 50% of the diet in summer. In their experiment, Muir et al. (2015) attributed the lack of milk production response of cows fed CH compared with RGWC on the stem material, which influenced DM, NDF, and metabolizable energy; and therefore, intake and milk production responses. Farmers may need to consider refusal of stems in feed allocation of second year chicory to avoid underfeeding. In the current experiment, total DMI did not differ between treatments, suggesting that the differences in milk response observed are explained by other factors such as grazing behavior, forage utilization, and/or nutritive value of the forages.

Cows increased their grazing intensity during the CH feeding periods (Figure 7.2). Chicory herbage consisted of 39% reproductive stem before grazing and 70% of reproductive stem after grazing, suggesting that the cows selected leaf over stem material. Clark et al. (1990b) reported enhanced animal performance at high leaf allowances from reproductive CH swards, a result similar to our experiment. The ratio of non-structural to structural carbohydrates was also greater for CH compared with RGWC herbage, which might have improved energy supply. Ruminants get nearly 70–80% of their energy supply from VFAs (Bergman, 1990). The mean concentration of total VFA was 10% greater for cows on CHAM and CHPM than those on RGWC were, which likely explains their increased milk production.

When comparing the two CH treatments, afternoon allocation of CH increased milk solid yield by 7.6% compared with the morning allocation. This reflects the greater fat percent ($p = 0.051$) and daily fat yield ($P < 0.001$) from cows offered CH in the afternoon than those offered CH in the morning and can be explained by the increased proportion of branched chain-VFA (iso-butyrate + iso-valerate) in the rumen of CHPM cows than CHAM cows (Figure 7.3). Increased proportion of branched chain-VFA in the rumen is associated with improved milk FA composition and milk yield in dairy cows (Liu et al., 2018). Although studies have looked into the timing of fresh herbage allocation and its effect on milk production (Orr et al., 2001; Abrahamse et al., 2009; Pulido et al., 2015), this is the first experiment to demonstrate the impact of timing of allocation of two different forage species on milk FA composition. The findings from the current study suggest that cows are more responsive to timing of allocation of herbage of some species (CH) more than others (RGWC) on milk yield and milk composition.

Another interesting observation arose from the evident synchrony between grazing behavior and rumen fermentation. Rumen VFAs, especially acetate and propionate, increased during peak grazing periods, with greater concentrations occurring when cows grazed CH than RGWC (Figure 7.3). The increase in the concentration of total VFA in rumen corresponded with declines in rumen pH in all treatment cows. pH values less than 5.8 are regarded as harmful to ruminal cellulolytic bacteria (Russell and Wilson, 1996), whereas pH less than 5.5 is said to be detrimental to the ruminal epithelium and VFA absorption in cows fed a high-concentrate diet (Gäbel et al., 2002). Cows fed RGWC were able to maintain their rumen pH above 5.8 likely because they ruminated more. Rumination increases saliva production rate and increases the supply of bicarbonate to the rumen to enhance total ruminal buffering capacity (Penner et al., 2010). Although ruminal pH was below 5.8 in cows offered CH between 1200 h and 2000 h in the current experiment, they were within a normal range of 5.6 to 6.4 previously reported in a review of 23 studies for dairy cows fed high-quality herbage (Kolver and De Veth, 2002). High-quality herbages are highly digestible and their ruminal fermentation is associated with increased VFA, but low lactic acid (de Veth and Kolver, 2001). Since VFAs provide most of the energy requirement of ruminants, it is not surprising that cows fed high-quality herbage diets produce more milk even at lower pH values (Penner et al., 2010).

7.4.2 Milk and rumen digesta FA composition

Our results confirm that, regardless of time of allocation, feeding CH at up to half the ration increases concentrations of beneficial FA; LA and ALA, in the milk of dairy cows compared with the traditional feeding regime of RGWC (Muir et al., 2014, 2015; Mangwe et al., 2018). The increase in the concentration of these is particularly important given their human health benefits. Alpha linolenic acid for example, has demonstrated potential to exert neuroprotective, anti-inflammatory, and antidepressant properties (Blondeau et al., 2015; Nguyen et al., 2019). de Goede et al. (2011) recently reported that increased ALA intake lowered the risk of stroke. In the body, ALA is converted to eicosapentaenoic acid, a FA that is known for its cardio-protective and other human health benefits (Rajaram, 2014). The concentration of these LA and ALA in milk mainly depends on their concentration in the diet and intake, level of biohydrogenation in the rumen, and amount absorbed in the duodenum (Elgersma, 2015). The higher concentration of LA in CH diets, compared with RGWC, is likely to explain its elevated concentration in the milk, though other mechanisms are also likely to be involved in the increased milk ALA concentrations, as concentrations in herbage were not different to the control. Mean concentrations of LA and ALA were 21% and 26% greater in the rumen digesta of cows grazing CH than those on RGWC, respectively, with peak concentrations occurring four hours after allocation of CH herbage (Figure 4). The greater concentration of LA and ALA in rumen digesta of cows grazing CH corresponded with lower concentration of VA and stearic acid. This suggests that the level of biohydrogenation was reduced when cows grazed CH, which increased their rumen outflow and subsequently their inter alia availability in the mammary gland, a similar premise shared by Szczechowiak et al. (2016) in milk of cows, fed condensed tannins and fish-soybean oil blend mixture.

There are two plausible explanations for the decreased biohydrogenation when cows grazed CH, with the first being due to lower pH. Lower ruminal pH is known to inhibit the activity of lipase thus limiting lipolysis (Chilliard et al., 2007). Given that lipolysis is a prerequisite for ruminal biohydrogenation, it is not surprising that more PUFA were recovered in milk of cows grazing CH. The other likely reason could be a faster rumen passage rate as a result of reductions in microbial contact with dietary FA (Lourenço et al., 2008; Elgersma, 2015). This premise is supported by the decreased concentration of odd and branched-chain FA in the rumen (Figure 7.4) and milk (Table 7.3) of cows grazing CH-based diets, as their lower concentrations in the rumen and milk of cows on CH indicate reduced microbial colonization of CH than RGWC herbage.

Milk from cows on RGWC had greater concentrations of VA (22% higher; P = 0.007) than cows on CHAM or CHPM (Table 7.2). Similarly, the RGWC regime elevated the concentration of CLA by 17% (P = 0.24) and 26% (P = 0.059) compared with cows on CHAM and CHPM feeding regimes, respectively. About 70% to 90% of CLA in the milk of cows originates from the oxidation of the precursor VA in the mammary gland and other tissues by enzyme $^{\Delta}9$ desaturase (Bryszak et al., 2018), hence, the strong relationship between the VA and CLA in cow milk. The concentration of VA was 2.6, 2.79, and 2.75 times that of CLA for cows grazing CHAM, CHPM, and RGWC, respectively in the current study, which is a little greater than the 2 to 2.5 reported by Elgersma et al. 2006).

7.5 Conclusions

Allocation of mature CH herbage to dairy cows at 50% of their ration improved both milk yield and the FA profile of the milk. Furthermore, offering CH in the afternoon compared with that in the morning increased the milk concentration and yield of desirable polyunsaturated fatty acids. Changes in milk yield were associated with increased utilization of high-quality leaf components of CH herbage compared with RGWC herbage. While changes in milk FA composition related to CH feeding, appear to be linked to reduced biohydrogenation of dietary FA, at lower rumen pH, which subsequently increased their concentration in milk of cows fed CH. Allocating CH herbage during the afternoon is a useful strategy that can translate to improved milk production and quality.

Chapter 8

8. Partial replacement of ryegrass and clover herbage with chicory to alter urination behaviour and soil nitrogen loading of grazing dairy cows

Part of this chapter is under review:

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Statement of Contributions of Joint Authorship

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Conceptualization, methodology, data collection, formal analysis, writing—original draft preparation.

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Conceptualization, methodology, review, editing and co-author of manuscript.

8.1 Introduction

Animal excreta, especially urine, is one of the major sources of nitrogen (**N**) contaminating the nearby agricultural waterways (Wachendorf et al., 2005). This is mainly because the N deposited in urine patches [200 to 2000 kg/ha N (Selbie et al., 2015)] is often at a rate too high for plants to use, resulting in N loss through leaching when drainage occurs (Scholefield et al., 1993; Wachendorf et al., 2005). Consequently, several strategies are being explored to mitigate N losses to achieve regulatory limits (Bryant et al., 2019). Reducing UN concentration has emerged as a promising strategy to reduce N loss in pastoral systems (Ledgard et al., 2015; Marshall et al., 2020). This is because the risks of nitrate leaching at urine patches are dependent on N load (Di and Cameron, 2002), which is reliant on urine volume and urinary N concentration [**UN**; (Li et al., 2012)].

Many studies favoured dietary features such as reduced forage crude protein content (Dijkstra et al., 2013), increased concentration of soluble carbohydrates to protein ratio (Edwards et al., 2007; Totty et al., 2013), increased concentration of minerals (Spek et al., 2012), and high moisture content (Box et al., 2017a) to reduce animal UN loading to soils. The use of forage herbs such as chicory (*Cichorium intybus* L.) to fully or partially replace the traditional ryegrass-white clover diet (RGWC: *Lolium perenne* L./ *Trifolium repens* L.). has been beneficial to reduce the concentration of UN in dairy cows (Minneé et al., 2017; Mangwe et al., 2019). This has been attributed to the high concentration of minerals such as sodium and potassium (Nennich et al., 2006; Spek et al., 2012; Ledgard et al., 2015), and/or the high moisture content of chicory, which increased total water intake **(TWI)** and urination frequency (Mangwe et al., 2019), resulting in more dilute urine, thereby lowering urinary N concentration.

While the impacts of chicory on urination frequency and UN concentration were quantified in the previous studies, the occurrence of the attributes that are relevant for chicory to reduce N losses (high moisture content) maybe distinctive for its vegetative stage. As an example, following vernalization in winter, chicory rapidly produces reproductive stems, which elongate quickly after bolting is initiated in late spring (Clapham et al., 2001; Mangwe et al., 2020c). The moisture content of stems of perennial herbaceous plants was described to be lower than that of leaves (Körner and Renhardt, 1987; Shipley and Vu, 2002). This could then reduce feed water intake

(FWI) to a point where TWI limits daily urine excretion output (Gregorini et al., 2018). Besides altering water metabolism, other factors like grazing behaviour (Gregorini et al., 2013) or a decline in CP content during maturing of chicory plants could play a role. To our knowledge, no data exists on urination frequency and urine N excretion patterns of dairy cows fed reproductive chicory forage.

On the other hand, while our first study demonstrated that sole diets of chicory elevated urination frequency and reduced UN concentration when compared with RGWC, it is less likely that farmers will feed animals 100% chicory for extended periods. The impacts of the chicory on urine excretion patterns may be smaller when its proportion in the diet is reduced (Bryant et al., 2018). By contrast, there is limited information on the best approach to include proportions of chicory into the pastoral system without sacrificing the potential environmental benefits. Numerous potential strategies exist to include the novel herbs into the traditional dairying system. They can be included in diverse pastures with grasses and legumes without reducing animal production (Totty et al., 2013; Bryant et al., 2017), or can be sown as monocultures within the farm (Glassey et al., 2013), an option which offers the opportunity to manage their individual requirements separately. Pembleton et al. (2016) showed that inclusion of spatially adjacent monocultures of forage herbs such as plantain or legumes such as white clover could improve animal production when compared with feeding RGWC. Similar studies that will further characterize the urination frequency and urinary N excretion patterns of cows grazing diets including proportions of monocultures of chicory are therefore strongly desirable to evaluate more widespread chicory adoption into the pastoral system.

Furthermore, other studies have shown that time of forage allocation could have impacts on animal performance (Gregorini, 2012; Garrett et al., 2019). These changes are associated with the variations in forage composition throughout the day (Pulido et al., 2015; Al-Marashdeh et al., 2016). For example, as photosynthates accumulate and water evaporates, the dry matter and non-fibre contents increase while crude protein and fibre contents of the forage decrease (Delagarde et al., 2000a; Bryant et al., 2014). The impacts of these changes during the day and the interaction between forages (in this case chicory vs RGWC), on urination behaviour, UN and rumen ammonia concentration have not been clearly established.

The main goal of the current grazing experiment was to obtain more information about the efficacy of high moisture forage chicory, particularly concerning the best time to allocate it with RGWC forage on diurnal patterns of urinations, UN and rumen ammonia concentration of mid lactating dairy cows in summer. The hypothesis of the study was that including chicory into the traditional grazing regime of RGWC would cause a differing response in urination frequency, UN and rumen ammonia concentration of mid lactating. Another hypothesis was that the response in the aforementioned parameters is different in cows offered chicory during the morning or the afternoon.

8.2 Materials and methods

Animals used and the study design were approved by the Lincoln University Animal Ethics Committee (AEC #2018-48). Thirty-six mid-lactating dairy cows in their 2-4 parities, 155 ± 3.3 days in milk, with an initial mean body weight of 483 ± 13.8 kg and milk yield 21.3 ± 0.97 kg/cow.day were evenly divided into nine groups (4 cows per group) and randomly allocated one of three replicated grazing regimes (4 cows per group). Grazing regimes were (a) RGWC; perennial ryegrass/white clover only, (b) CHAM; ryegrass/white clover plus morning allocation of CH and (c) CHPM; ryegrass/white clover + afternoon allocation of CH. One cow per group had previously been fitted with a rumen cannulae (Bar-Diamond; Parma, Idaho, USA).

8.2.1 Management and grazing regimes

The study carried out at the Lincoln University Research Dairy Farm, Canterbury, New Zealand was a part of a larger study that investigated effects feeding vernalised CH on rumen fermentation and fatty acid profile of milk of mid lactating dairy cows, and had been described in more detail previously (Mangwe et al., 2020b). This study, conducted between 10 December 2018 and 25 January 2019, included a four-week baseline measurement period in which chicory cows were offered RGWC plus 15-20% CH forage, a four-day adaptation period in which the relative proportion of chicory was increased to 50% of the diet, and an eleven-day measurement period. During the study, all animals received a fresh forage allocation of 34 kg DM/cow per day above ground. The area to be grazed each day was adjusted based on forage mass estimated every three days by cutting all forage within three 0.25-m^2 quadrats in each treatment replicate to ground level using an electric hand-piece in the area immediately next to the area currently grazed. All cut forage was washed prior to oven drying at 60 °C to a constant weight and weighed to calculate pre-graze forage mass (kg/ha DM). Cows on RGWC only were allocated a new RGWC forage (34 kg of DM per cow) after each morning milking. Cows on CHAM were allocated a new CH forage (17 kg of DM per cow) after morning milking and a new RGWC forage (17 kg of DM per cow) after afternoon milking. Cows on CHPM were allocated a new RGWC forage (17 kg of DM per cow) after morning milking and a new CH forage (17 kg of DM per cow) after afternoon milking. Cows on CHPM were moved back to RGWC forage at 2130 hrs to ensure that all cows on CH treatments spent equal time (5 hours) on CH forage.

8.2.2 Herbage sampling and analyses

Pre-grazing forage samples were collected to ground level on the mornings and the afternoons' allocations before the cows were moved into their new allocations (0700 h and 1300 h, respectively) on days 6, 8 and 11 of the study. Post-grazing samples were collected the following morning after each grazing event. Collected samples were composited and subsampled for dry matter (DM) determination. The DM content of the forage was determined immediately by drying the samples at 60 ˚C for 48 h in a hot air oven. The remaining samples were freeze dried, ground to pass a 1-mm screen (ZM200 Retsch) and analysed for Organic matter (OM), water-soluble carbohydrates (WSC), neutral and acid detergent fibre (NDF, ADF) and crude protein (CP) by using near-infrared spectrophotometry (NIRS, Model: FOSS NIRSystems 5000, Maryland, USA) as previously described (Mangwe et al., 2020b).

Forage DM intake for each forage type for cows in each group was estimated on day 6, 8 and 11 of the study from the difference between pre- and post-grazed forage mass as follows;

$$\text{DMI} = (\text{pre graze mass (kg DM/ha)} - \text{ post graze mass (kg DM/ha)}) \times \text{area (ha)} \div \text{no. of animals}$$

Apparent N intake per cow per day was estimated by multiplying the pre and post forage mass by the N content of the pre and post graze forage. Water troughs in each paddock were fitted with flow meters to record daily drinking water intake by each group.

8.2.3 Milk urea nitrogen measurements

All cows were milked twice daily at around 0700 and 1400 h with an automated system (DeLaval Alpro Herd Management System, DeLaval, Tumba, Sweden). During milking, on days 6, 8 and

11 milk samples were collected from each cow during an AM and PM milking for milk urea nitrogen (MUN) determination. To analyse for MUN, the collected milk samples were centrifuged at 4000 g for 10 minutes at room temperature and refrigerated for 10 min to allow the fat to solidify on the top and be removed. Milk urea N was then determined on skimmed milk by an automated Modular P analyser (Roche/Hitachi) (Talke and Schubert, 1965).

8.2.4 Urination behaviour measurements

Lincoln University PEETER sensors were used to measure urination behaviour of the cows. Details of the design of the sensor and attachment to cows were previously outlined by (Mangwe et al., 2019). Briefly, a sensor sleeve is attached over the vulva of the cow using a ventilated 3D printed mould secured by a biocompatible glue. A pressure sensor is attached at the bottom of the sleeve. During each urination event, urine is channelled to pass through the sensor, which records the volume based on laboratory calibrations. The urine then exits through a small hall located at the bottom of the sensor, thus emptying the chamber for subsequent urination events.

Urination behaviour was measured from 6 cows daily per treatment between days 7 and 13 of the study. At least one cow per group wore the sensor during each run. Three cows per treatment wore the sensor on day 7 immediately after morning milking and spot urine sampling. The sensors remained on the cows until the afternoon milking of day 9 of the study (± 52 hours). The urine harnesses were removed to enable urine and faecal spot sampling. Urine sensors were again attached to a different set of 3 cows per treatment following the morning milking of day 10 and were removed following the morning milking of day 13 (± 75 hours). Sensors were monitored during each milking, and rechargeable batteries were replaced after 36 hours. To minimize time spent in the yard when replacing batteries, sensors were detached during milking and re-attached immediately after milking. A harness was removed if it showed signs of becoming detached. Consequently, a complete set of 72-h data was collected from 7 cows (2 from RGWC, 3 from CHAM and 2 form CHPM) and another set of 24-h data was collected from 8 cows during the study (2 from RGWC, 3 from CHAM and 3 from CHPM).

8.2.5 Urine and dung

Spot urine and dung samples were collected from all 36 cows following the morning and afternoon milking of days 6 and 9 of the study. Additional urine samples were collected from 9 ruminal cannulated cows (three cows per treatment) at four-hourly intervals starting from 0400 to 2400 h on day 13 and 15 of the study. Urine samples were collected by manual stimulation of the area beneath the vulva while dung was collected via rectal grab. Faecal samples were stored frozen at −20 °C immediately after collection, while urine samples were acidified with sulphuric acid to prevent N volatilisation and stored at −20 °C pending further analysis. The stored faecal and urine samples were analysed for N concentration by combustion (Vario MAX CN, Elementar Analysensysteme, Hanau, Germany). Urine urea concentration was determined using a commercial enzymatic kinetic technique (Randox, Crumlin, Co., Antrim, UK). Creatinine concentration was determined by the Jaffe method (Daytona RX Clinical Analyser, Randox, Nishinomiya, Japan).

8.2.6 Rumen sampling and rumen ammonia analysis

Nine cows fitted with the rumen fistula were utilised to determine the rumen ammonia concentration. Rumen digesta samples were collected at four-hourly intervals starting from 0400 to 2400 h on day 13 and 15 of the study by hand, via the rumen fistula, from the mid and dorsal rumen where fermentation is most active. Rumen fluid samples were collected by squeezing a subsample of the composited rumen digesta through two layers of cheesecloth. The collected samples were acidified with sulphuric acid to prevent N volatilisation and stored at −20 °C pending ammonia analysis. Ammonia was analysed using an enzymatic UV method using a Randox ammonia kit and the Randox Rx Daytona analyser (United Kingdom).

8.2.7 Statistics and calculations

All data were analysed using R (R Core Team, 2018). Animal group was used as an experimental unit for all statistical analysis. Milk urea nitrogen, urine and faeces data for spot samples collected from all 36 cows were analysed using mixed-effects model in R using the lme4' package version 1.1-21 of R (Bates et al., 2015) with the models containing grazing regime (CHAM, CHPM, and RGWC) as fixed effect, while animal nested in sampling day used as random effect. For urine samples taken at 4-hourly intervals, grazing regime was included as fixed effect, sampling time

(0400, 0800, 1200, 1600, 2000, 2400 h) as a repeated measure, while sampling day used as random effect. To determine the effects of feeding regime on daily urination patterns (time between urination events/cow, total number of urinations per day, total urine volume/cow.day and urine volume/event) we selected cows with a minimum set of 24-hour data. The models contained grazing regime as a fixed effect while animal nested on day (day in this case refers to a 24-hour cycle) used as random effect. Means were separated using the ‘emmeans’ package of R. The significant differences were tested with Tukey post-hoc test ($P < 0.05$).

8.3 Results

Climate conditions were dry during the study except for 2- and 3-mm rain on day 2 and 4, respectively, of the study. The minimum temperatures ranged from 8.6 to 14.3 with an average of 11.5, while maximum temperatures ranged from 17.8 to 30.1, with an average of 22.7^{o}C. Sunrise occurred between 0607 and 0620 h and sunset at 2105 h and 2109 h, with an average day length of 14.8 hours.

8.3.1 Chemical composition

The chemical composition of chicory and RGWC is presented in Table 8.1. There was a treatment effect for DM, OM, CP, NDF and NFC ($P \leq 0.015$). Dry matter, CP and NDF were 18.2%, 19.3% and 14.4% greater for RGWC than CHAM or CHPM treatments, while NFC was 37% greater for CHAM and CHPM than RGWC treatments. When comparing the two forages, chicory had greater NFC, but less DM, OM, CP, NDF, and ADF contents than RGWC ($P < 0.05$). The concentration of DM and NFC was greater for samples taken during the afternoon than those taken during the morning, irrespective of forage type ($P < 0.05$).

Table 8.1**.** Pre-grazing chemical composition of chicory and ryegrass/white clover sampled to ground level.

Herbage	**Chicory herbage**		**Ryegrass/white clover herbage**			**SEM[4]**	**P - value**		
Treatments	**CHAM[1]**	**CHPM[1]**	**CHAM[1]**	**CHPM[1]**	**RGWC[1]**		**Treatment (T)**	**Herbage (H)**	**T x H**
Dry matter (g/kg DM)	112	129	211	196	198	6.6	0.003	< 0.001	0.221
Organic matter (g/kg DM)	875	895	923	916	918	4.6	0.015	< 0.001	0.113
Water soluble carbohydrates (g/kg DM)	154	232	222	200	193	18.2	0.332	0.321	0.019
Crude protein (g/kg DM)	149	119	152	167	182	9.9	0.011	0.095	0.153
Neutral detergent fibre (g/kg DM)	312	258	446	480	437	12.8	0.004	< 0.001	0.006
Acid detergent fibre (g/kg DM)	248	222	262	269	25.4	11.3	0.675	0.021	0.166
DOMD[2] (g/kg DM)	702	742	740	704	740	10.4	0.344	0.986	0.005
NFC[3] (g/kg DM)	396	457	285	243	252	18.4	0.004	< 0.001	0.019

[1]RGWC = perennial ryegrass/white clover only; CHAM = ryegrass/white clover + morning allocation of chicory; CHPM = ryegrass/white clover + afternoon allocation of chicory. [2]DOMD = Digestibility of the organic matter in the dry matter. [3]NFC = Non-fibre carbohydrates (1000- (NDF + CP + Fat +Ash)). [4]SEM = standard error of the mean.

8.3.2 Grazing behaviour

Total daily grazing (499 ± 15 min/cow per day) did not differ between treatments, while total daily time ruminating was highest for RGWC (446 ± 9.34 min/cow per day), intermediate for CHAM (379 ± 9.69 min/cow per day), and lowest for CHPM (359 ± 10.13 min/cow per day) cows $P < 0.001$). Table 8.2 presents the intensity (min/h) of grazing and rumination of dairy cows offered RGWC or chicory-based diets in this study. Grazing intensity was influenced by whether cows received chicory and when they received it. Cows on RGWC had similar grazing intensity in the morning and afternoon while chicory fed cows had greater grazing intensity depending on whether they received chicory in the morning or afternoon. During the morning grazing bout (0900 – 1330 h), the intensity of grazing was 1.3 and 2.0 times greater for cows on CHAM than those cows on RGWC and CHPM, respectively. While during afternoon grazing bout (1700 – 2130 h), grazing intensity was 1.2 and 1.4 time greater for cows on CHPM than those cows on RGWC and CHAM, respectively.

Table 8.2**.** Effect of grazing regime on diurnal variation of time spent grazing and time spent ruminating (min/h).

	Treatments[1]				
	CHAM	CHPM	RGWC	SEM	P - value
Grazing					
AM milking (0600-0800 h)[2]	21.2	16.3	22.5	3.98	0.264
AM Grazing (0900-1330 h)	45.5^{a}	23.2^{c}	34.5^{b}	3.08	0.001
PM Milking (1400-1630 h)[2]	16.1	14.1	15.3	4.87	0.988
PM Grazing (1700-2130 h)	30.7^{b}	42.2^{a}	35.1^{b}	3.08	0.001
Night (2200-0600h)	4.8	7.2	4.8	2.30	0.551
Rumination					
AM milking (0600-0800 h)[2]	7.3	8.0	7.7	2.82	0.995
AM Grazing (0900-1330 h)	2.0^{c}	15.1^{a}	7.2^{b}	2.18	0.001
PM Milking (1400-1630 h)[2]	8.5	15.1	12.4	2.44	0.139
PM Grazing (1700-2130 h)	7.5^{a}	0.9^{b}	10.7^{a}	2.18	<.0001
Night (2200-0600h)	33.8^{a}	24.4^{b}	33.5^{a}	1.63	<.0001

$^{a–b}$Means within a row with different superscripts differ ($P < 0.05$).

[1]RGWC = perennial ryegrass/white clover only; CHAM = ryegrass/white clover + morning allocation of chicory; CHPM = ryegrass/white clover + afternoon allocation of chicory.

[2]Includes time when cows were moved to the milking shed, milking and drafting.

8.3.3 Water balance and urination behaviour

Including chicory into the traditional grazing regime (CHAM, CHPM) increased total TWI by 21.1 L/day compared with the control RGWC ($P = 0.043$; Table 8.3). This increase was explained by FWI, which accounted for 76, 78 and 64% of TWI for cows on CHAM, CHPM and RGWC, respectively. As with TWI, daily urine volume was greater by 21.2 L/cow.day for cows offered chicory (CHAM, CHPM) compared with that of cows offered RGWC ($P < 0.001$). Urine volume per event varied between 0.25 – 7.8 L per urination event (Figure 8.1A), with an average of 3.00 ± 0.3 L/cow.event (Table 8.3; $P = 0.71$). Including chicory increased urination frequency, averaging 15.7, 17.7 and 9.9 ± 1.12 events/cow.day for cows on CHAM, CHPM and RGWC, respectively ($P < 0.001$). While faecal DM content was not different between treatments (87.7 g/kg DM), faecal water was estimated to be 31.9 L/cow.day greater for cows offered RGWC compared with that of cows offered chicory (CHAM, CHPM; $P < 0.05$).

Table 8.3**.** Means of variables used for estimating water balance in cows offered chicory and ryegrass/white clover.

Item	**CHAM[1]**	**CHPM[1]**	**RGWC[1]**	**SEM[2]**	**P - value**
Water intake					
from troughs (L/cow.day)	28.4^{b}	25.1^{b}	35.9^{a}	1.17	<0.001
from feed (L/cow.day)	92.2^{a}	91.3^{a}	62.3^{b}	3.79	0.008
Total water intake (L/cow.day)	121.0^{a}	117.1^{a}	98.1^{b}	3.63	0.043
Water in milk (L/cow.day)	18.1^{a}	18.9^{a}	17.2^{b}	0.48	0.001
Urine volume per event	3.14	2.91	2.95	0.31	0.710
Urinations per day (events/cow.day)	15.7^{a}	17.7^{a}	9.9^{b}	1.12	<0.001
Time between urination events (min)	92.4^{b}	92.2^{b}	144.1^{a}	8.42	<0.001
Urine volume per day (L/cow.day)	48.5^{a}	48.1^{a}	28.1^{b}	3.11	<0.001
Faecal DM (g/kg DM)	88.9	87.8	85.5	1.7	0.690
Faecal water output (kg/cow.day)[3]	24.8^{b}	24.1^{b}	51.4^{a}	12.7	0.021

$^{a–b}$Means within a row with different superscripts differ ($P < 0.05$).

[2]SEM = Standard error of the mean.

[3]Faecal water output (kg/day) = (Faecal DM output × [(100 – faecal DM percentage) / faecal DM percentage], where

Faecal DM output (kg/day) = 0.43 × DMI – 0.0000198 × CP of forages – 2.30 (Khelil-Arfa et al., 2012).

Diurnal patterns of urination events per cow are presented in Figure 8.1. The pattern of urination intensity was the same, with more urination events during intensive grazing bouts, but the density of urination events was different among the treatments. This increase in urination events during intensive grazing was more obvious when cows had grazed chicory (Figure 8.1).

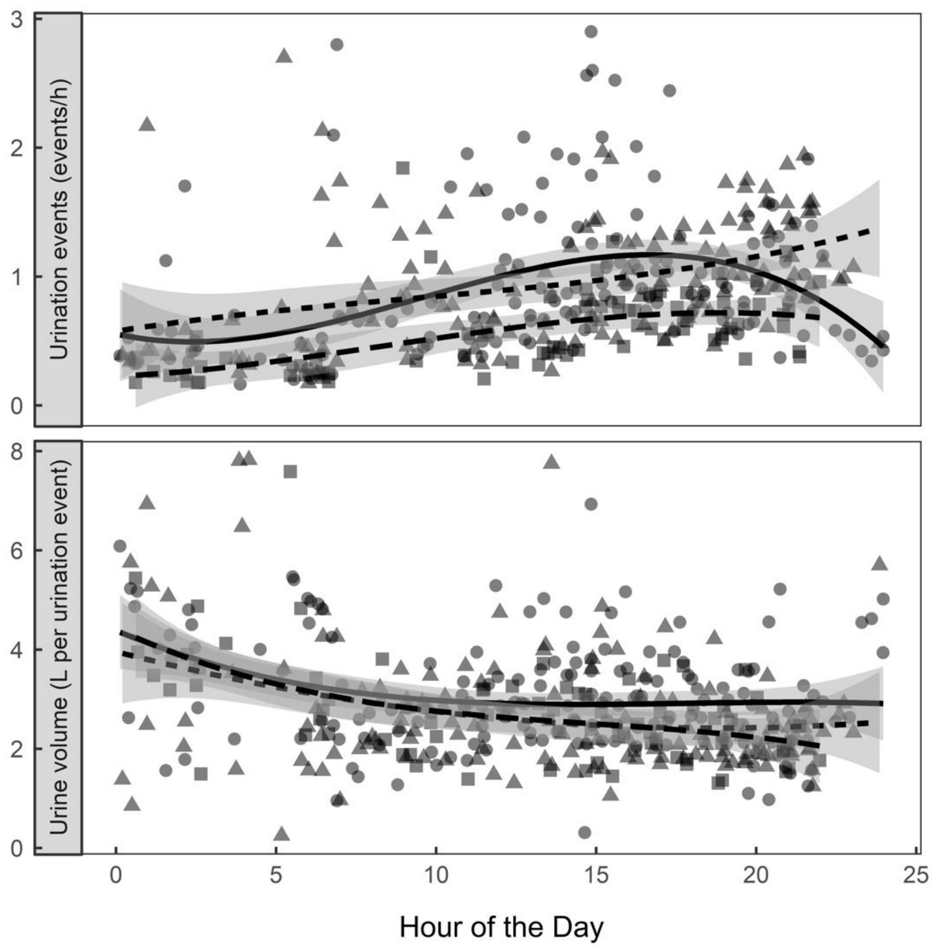

Figure 8.1. Diurnal variation of urination events per hour and urine volume as influenced by feeding regime. Square points and long dashed line points denote RGWC (perennial ryegrass/white clover only), triangle points and solid line denote CHAM (ryegrass/white clover + morning allocation of chicory) while circle points and short dashed denote CHPM (ryegrass/white clover + afternoon allocation of chicory). Each point represents a single urination event.

On closer inspection (Figure 8.2), urination events increased ($P < 0.05$) by 40% between 0800 – 1200 h, and by 60% between 2000 – 2400 h for cows offered CHPM compared with cows offered

RGWC. Cows offered CHAM increased (P < 0.05) urinations events by 41% between 1200 and 1800 h when compared with cows offered RGWC. When comparing the two chicory treatments, cows offered CHAM had more urination events than cows offered CHPM between 1300 – 1900 h (+21%), with a tendency to urinate more frequently detected between 1300 – 1500 h (0.82 vs 0.59 events/cow.h; P < 0.1). Whereas cows offered CHPM had more urination events during the night and morning hours (+31%) compared with CHAM, with a tendency to urinate more frequently detected between 2100 – 2400 h (0.95 vs 0.62 events/cow.h; P < 0.1).

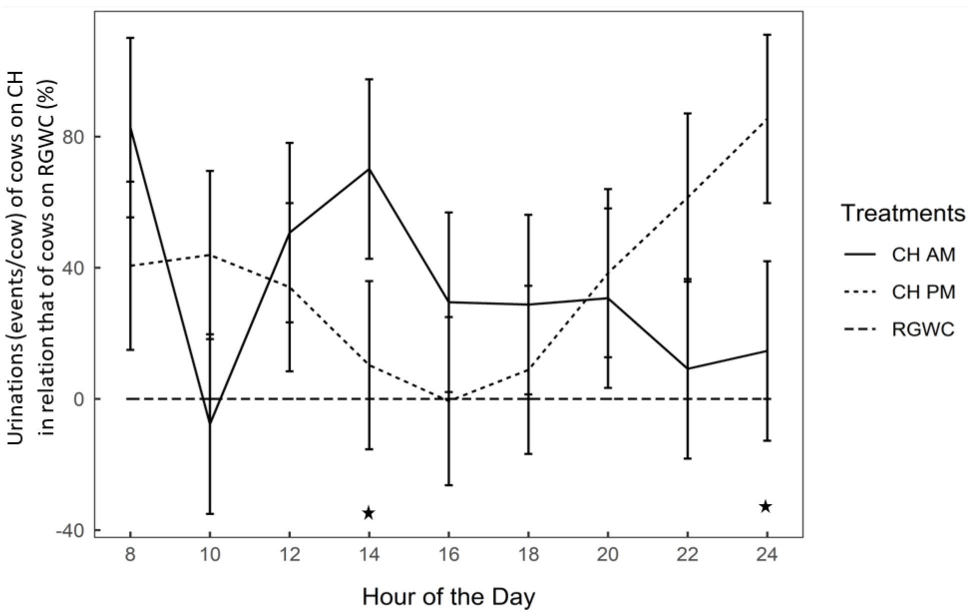

Figure 8.2**.** Effect of inclusion and time of allocation of chicory on urination events per cow averaged in 2 hr intervals following allocation of fresh herbage (~0800 and 1600 hrs for morning and afternoon allocations, respectively) in relation to the number of urination events of the control cows on RGWC measured at the same time of the day. Urination events of cows on RGWC only were set to 0%. CHAM (ryegrass/white clover + morning allocation of chicory), CHPM (ryegrass/white clover + afternoon allocation of chicory), RGWC (perennial ryegrass/white clover only). Solid lines denote CHAM, short dashed lines denote CHPM and long dashed line denote RGWC. Data are displayed as arithmetic means ± standard deviation. The asterisks indicate tendencies at P ≤ 0.1 between measurements made at the same time for cows fed CHAM vs CHPM.

8.3.4 Nitrogen utilization

The average CP concentration of pre grazed chicory was low at 13.4 compared with 16.7% ± 0.9 % of DM in RGWC herbage ($P = 0.095$). However, apparent N intake was unaffected by treatment (Table 8.4; 623 ± 36.5 g/cow.day; $P = 0.151$). Cows offered chicory had 21% lower ($P < 0.001$) MUN than the control cows, regardless of time of chicory allocation. Daily milk N and N use efficiency output were greatest for cows on CHPM, intermediate for cows on CHAM and lowest for cows on RGWC ($P \leq 0.03$). Estimated total daily urine N excretion was not different between CHPM and RGWC (199 ± 12.7 g/cow.day) but were greater ($P = 0.081$) than CHAM cows (168 ± 12.7 g/cow.day; Table 8.4).

The increase in urination events of cows offered chicory was associated with a decrease in the concentration of UN during the day, which averaged 3.6 g/L, 3.8 g/L and 5.5 g/L for cows on CHAM, CHPM and RGWC treatments, respectively. Mean N load was 11.8, 12.1 and 20.0 ± 1.4 ($P < 0.001$) for cows on CHAM, CHPM and RGWC treatments, respectively. There was no effect of forage treatment on faecal N concentration (31.1 g/kg of DM; $P = 0.69$) and estimated total faecal N output (122 g N/cow.day; $P = 0.131$).

Diurnal patterns of UN as influenced by grazing regime were observed with peak UN concentration in samples taken at 1200 hours, while lowest concentrations were observed in samples taken between 2000 and 0400 hours. Urinary N concentration was the same for cows offered CHAM or CHPM, but 43% lower than cows offered RGWC during morning peak grazing at 1200 h (0.44 vs 0.77 ± 0.18 g/kg; $P < 0.05$). A further decline in UN concentration was detected following greater urination activity, with cows offered CHAM having lower UN concentration than cows offered CHPM (0.23 vs 0.42 ± 0.11 g/kg $P \leq 0.05$) at 2000 h.

As with UN concentration, N loading rates were generally lower for cows on chicory than those on RGWC (Figure 8.3). Cows offered CHAM had consistently lower N load than RGWS between 1600 and 2000 hours ($P \leq 0.074$, while N load from CHPM was lower during the evening and dawn, with significant differences detected in samples taken at 0400 hours (8.7 vs 28.6 g N/event; $P = 0.003$).

Table 8.4. Apparent nitrogen (N) intake, spot urine composition and faecal N concentration from cows offered chicory and ryegrass/white clover.

Items	CHAM[1]	CHPM[1]	RGWC[1]	SEM[4]	*P* - value
N intake (g/cow.day)	646	556	667	36.5	0.151
Milk urea (mmol/L)	3.08[b]	3.39[b]	4.08[a]	0.11	<0.001
Milk urea nitrogen (mmol/L)	6.16[b]	6.77[b]	8.15[a]	0.22	<0.001
Milk N output (g/cow.day)	127[a]	129[a]	115[b]	3.14	<0.001
N use efficiency (g/g N eaten)	0.19[b]	0.24[a]	0.17[b]	0.89	0.025
Urine N (g/ L)	3.6[b]	3.8[b]	5.5[a]	0.3	<0.001
Urine Urea (mmol/L)	77.9[b]	89.6[b]	139[a]	7.52	<0.001
Urine NH_3 (mmol/L)	0.70[b]	0.73[b]	1.32[a]	0.12	0.001
Creatinine (mmol/L)	1.65[b]	1.65[b]	2.14[a]	0.15	0.030
Urine N output (g/cow/day)[2]	168	198	200	12.7	0.081
Faecal N (g/kg DM)	31.8	31.3	30.2	1.12	0.583
Faecal N output (g/cow/day)[3]	121	119	127	3.45	0.131

[a–b] Means within a row with different superscripts differ ($P < 0.05$).

[1]RGWC = perennial ryegrass/white clover only; CHAM = ryegrass/white clover + morning allocation of chicory; CHPM = ryegrass/white clover + afternoon allocation of chicory.

[2]Total daily urine output was estimated based on urine N concentration from 4 hourly spot samples and total urine volume of the 4 hours preceding the spot sampling. Average urine and faecal concentration were derived from spot samples collected from all 36 cows post morning and afternoon milking.

[3]Faecal N output was estimated as Faecal N = - 4.623 + (N_{diet} × 1.970) + (DMI × 7.890) (Pacheco et al., 2018).

[3]SEM = standard error of the mean.

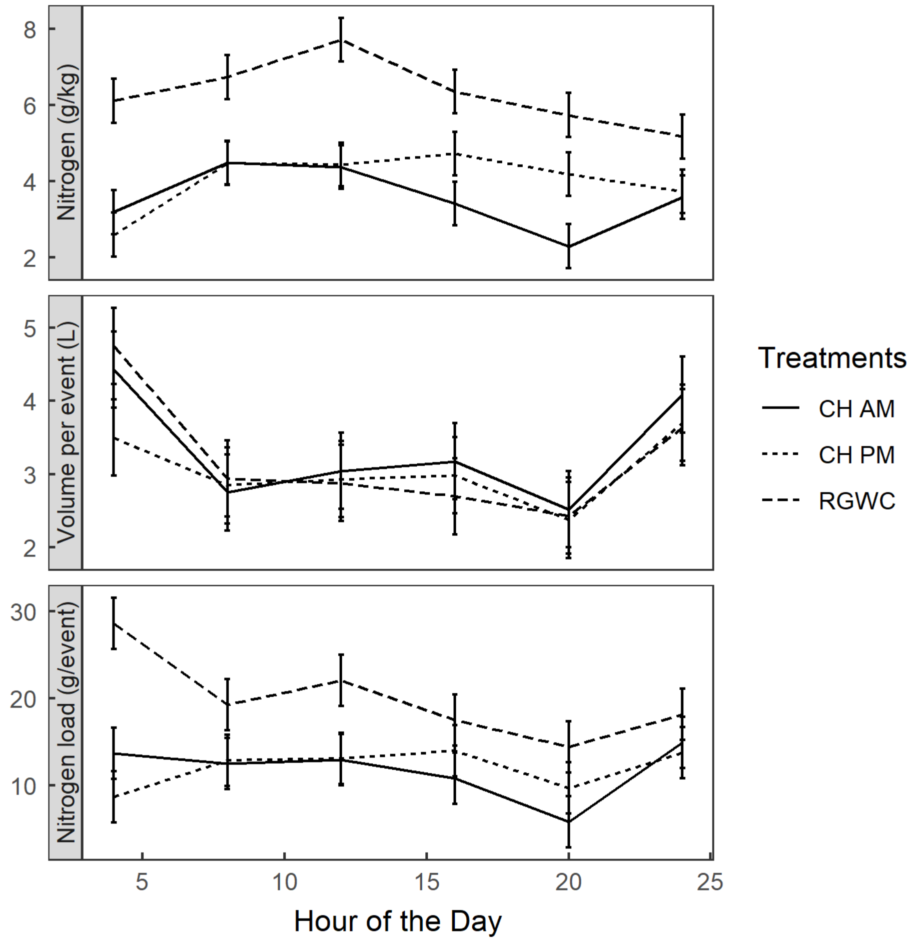

Figure 8.3. Diurnal variation of urine N composition, urine volume per event and N load. CHAM = ryegrass/white clover + morning allocation of chicory), CHPM = ryegrass/white clover + afternoon allocation of chicory and RGWC perennial ryegrass/white clover only. Error bars are standard error of the mean (n =3).

8.3.5 Rumen ammonia concentration

Mean concentration of rumen ammonia was lower for RGWC and CHAM cows (9.54 and 10.7 ± 1.38 mmol/L, respectively) than CHPM cows (11.8 ± 1.42 mmol/L, P = 0.007). There was a treatment and time of day interaction for rumen ammonia concentration (Figure 8.4; P = 0.0004). Rumen ammonia concentration increased (P < 0.05) shortly after allocation of fresh forage, with cows offered CHAM or CHPM having 1.8 times greater ammonia concentrations than the cows offered RGWC at 2000 hrs (P = 0.0004).

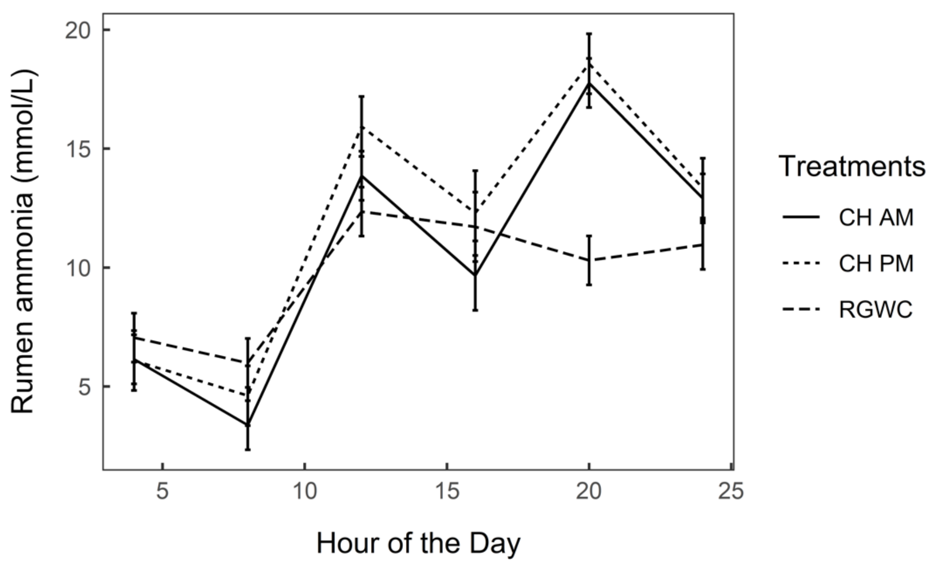

Figure 8.4. Effect of grazing regime: CH AM (ryegrass/white clover + morning allocation of chicory), CH PM (ryegrass/white clover + afternoon allocation of chicory), RGWC (perennial ryegrass/white clover only) on rumen ammonia concentration (mmol/L) sampled at 4-hourly intervals during the day between 0400 – 2400 hrs). Solid lines denote CHAM, short dashed lines denote CHPM and long dashed line denote RGWC. Error bars are standard error of the mean (n =3).

8.4 Discussion

The present study tested the hypothesis that including chicory into the traditional grazing regime of RGWC will cause differing responses in urination frequency, UN and rumen ammonia concentration of mid lactating dairy cows. Also, we hypothesized that the responses in the aforementioned parameters are different in cows first offered chicory during the morning or the afternoon. These hypotheses was based on the expectation of a changing water intake with feeding chicory, as previously reported for high moisture forage herbs (Box et al., 2017a; Mangwe et al., 2019), and the known diurnal fluctuations in forage composition throughout the day (Delagarde et al., 2000a). We anticipated that including vernalised chicory would lead to elevated TWI and urination frequency, diluting the concentration of N in the urine compared with control diets of ryegrass and white clover. Our results supported this hypothesis. When comparing the two chicory treatments, time of day had effects on diurnal patterns of urination frequency and UN, but not their mean values/or daily totals.

8.4.1 Effect of grazing regime on daily water intake, urine and faecal water output

Our findings supported the hypothesis that including chicory (CHAM, CHPM) in a RGWC diet of dairy cattle would increase TWI, a result that was attributed to greater FWI from the herb (Table 8.3). Apparent DMI did not differ among treatments, suggesting that the differences in FWI observed are explained by the DM% of the herbages. As DM% of forages increases, drinking water increases, but TWI decreases (Murphy, 1992). That observation is supported by the results from the current study, as cows offered RGWC drank 25% more water from the troughs, but TWI was 17.5% lower than that of cows offered chicory (CHAM, CHPM). Drinking and FWI are sufficient to provide 80 to 90% of the daily water needs of lactating dairy cows (Murphy, 1992). In the current experiment, estimated water requirements per day were 61, 62 and 60 L/cow.day for cows offered CHAM, CHPM and RGWC, respectively (NRC, 2001); therefore the range of 98 – 121 L/cow.day for all cows in the study was surplus to requirements.

Cows fed high moisture diets meet their water requirements by increasing the loss of water in faeces and urine (NRC, 2001). Appuhamy et al. (2014) predicted faecal water to account for an average of 61% of the total manure water output from lactating dairy cows. In agreement, our results showed that cows offered the control RGWC diet contributed 64% to the total manure water output (urine plus faecal water). This was greater than the ~33% contributed by cows offered chicory. This suggested that cows offered chicory excrete more water via urine, confirming the diuretic properties of chicory (Chapter 3). While the effect of chicory is slightly less than that from the previous study when chicory was fed at 100% of the diet (Chapter 3), both studies have consistently shown increased urine output from cows offered chicory diets compared with those offered RGWC only. Together with previous studies (Box et al., 2017a; Bryant et al. 2018), our results demonstrate that high moisture forage crops alter urine excretion patterns, which ultimately provides useful information for predicting urine output of cows under pastoral systems. This is particularly important because evaluation of potential contribution of alternative forages to N loss is likely to be carried out through modelling, which requires confidence in tools which are used to predict N loss.

Several studies have looked into the proportion of urinary water in relation to TWI. Khelil-Arfa et al. (2012) predicted urine volume to account for 24% of TWI. Using a different sensor, Bryant et al. (2018) reported urine volume to account for 26% of TWI from cows grazing RGWC. In a recent total collection study of lactating dairy cows offered a corn silage-based diet and an alfalfa silage-based diet, total urine excreted accounted for 27-34% of TWI (Lee et al., 2018). These values are not different from the 29% for cows on RGWC, but lower than the 40% for cows on chicory in the current study. Though greater than those on RGWC, the proportion of urine water to TWI for cows on chicory is near the 42-50% reported for plantain (Box et al., 2017a) and non-vernalised chicory (Chapter 3) reported previously, lending further support to water diuresis effects of the two forage herbs (O'Connell et al., 2016). Milk water, on the other hand, accounted 15-16%, and 18% of the water ingested for cows on chicory and those on RGWC, respectively. We did not determine water loss in faeces during our study, but estimates based on Khelil-Arfa et al. (2012) equations showed that faecal water accounted for approximately 21% of the TWI for cows grazing chicory, and 52% for cows grazing the control RGWC herbage. While formulas for estimating fecal water from cows grazing RGWC resulted in water balance, the same formula for fecal water loss from cows grazing chicory resulted in 27.8 L/cow.day which could not be accounted for in urine, faecal or milk water.

8.4.2 Effect of grazing regime on urination patterns

The total number of urination events of 9.9 events/cow.day for cows offered RGWC in the current study are at the lower end of the normal range of 9–14.1 events/day for dairy cows grazing grass diets in New Zealand (Selbie et al., 2015). The increased number of urination events for cows on chicory (CHAM, CHPM) compared with those on RGWC collaborate earlier findings from a review of trials using dairy cows grazing high moisture forage crops (Bryant et al., 2019). Studies using urine sensors have also reported diurnal patterns in urination patterns of dairy cows (Shepherd et al., 2017; Bryant et al., 2018). The pattern of events is reportedly the same for different forages (Chapter 3), with more urination events during high activity [feeding and milking; (Aland et al., 2002; Draganova et al., 2016)]. However, our findings further showed that the density of urination events was greater when cows had grazed chicory than when grazed RGWC herbage (Figure 8.1). For example, more events (+ 45%) for cows offered chicory during the morning (CHAM) than those offered CHPM and RGWC were recorded between 1200 – 1600 h. While, more urination events (+ 46%) for cows offered chicory during the afternoon (CHPM) than those offered CHAM and RGWC were recorded between 2000 – 2400 h (Figure 8.2). Temporal factors affecting the frequency of urinations include temperature (Draganova et al., 2016), activity (Aland et al., 2002), rumen fill (Noh et al., 2011; Gregorini et al., 2018) and urine osmolality (Maltz and Silanikove, 1996). The cows were observed to start grazing immediately after being allocated chicory and increased their grazing intensity (min/hr) during the chicory feeding periods compared with during RGWC feeding periods (Table 8.2). The increase in urination events likely resulted from the increased rumen liquid during intensive chicory grazing periods, which might have increased the frequency and volume of water flowing to the bladder (Gregorini et al., 2018), resulting in greater urination events. The consistency and repeatability of the patterns of urination events observed for cows offered chicory at different times of the day provide more information about the efficiency of chicory, particularly concerning the best time to allocate it with RGWC forage on diurnal patterns of urinations.

8.4.3 Effect of grazing regime on N excretion

The results from the current study showed that offering chicory during the morning grazing bout reduced the total urinary N by nearly 16% when compared with the traditional RGWC herbage or CHPM. Reductions in total urinary N are desirable as they diminish N losses from pastoral systems. These findings are consistent with earlier findings which also reported reductions in total urinary N output from cows offered diverse swards containing chicory (Totty et al., 2013; Bryant et al. 2017). The decrease in estimated N excretion in the previous studies was linked to lower CP content and N intake from the diverse swards. In the current study, apparent N intake was not statistically different among treatments, suggesting that other mechanisms that reduced total N output from CHAM cows maybe involved.

A possible explanation for the decrease in urinary N output from cows on CHAM could be improvements in N use efficiency of cows fed CHAM. This is supported by the lower concentration of MUN, a biomarker of the efficiency for milk production in lactating cows (Huhtanen et al., 2015). At similar apparent N intake, improvements in N use efficiency for milk production and reductions in UN concentration suggest that N partitioning was altered in cows offered chicory, a similar premise offered by (Totty et al., 2013) for cows consuming diverse diets containing chicory. Chicory forage has a higher ratio of non-structural carbohydrates to crude protein than RGWC (Minneé et al. 2017) which might have improved energy and protein supply to meet microbial requirements and improved milk protein daily yields and N use efficiency (Chapter 7).

Despite similar estimates for total N excretion between CHPM and RGWC, UN concentration was lower for both CHPM and CHAM than RGWC cows. A likely reason for the decline in UN concentration from cows on chicory forage is the increased urination frequency, which diluted the N content of the urine. Dilution of urinary N by increased urination frequency has been proposed as a means for reducing UN concentration and in cows fed high moisture forage diets (Bryant et al., 2019). In some studies, mineral supplements such as sodium chloride have been fed to cows to increase water intake to dilute the urine (Spek et al., 2012; Ledgard et al., 2015). The decrease in UN concentration from cows grazing chicory is particularly important because risks of nitrate leaching at urine patches depend on N load, which is a function of urine volume and concentration of UN (Li et al., 2012). As urine volume per event was not affected by feeding regime, chicory feeding reduced N loading at patch level, regardless of time of its allocation (Figure 8.3). Extrapolations based on the equations developed by Selbie et al. (2015) to estimate the average UN loading rate;

$$\text{UN rate (kg UN ha}^{-1}) = \text{Conc (g UN L}^{-1}) \frac{\text{Vol (L)}}{\text{Area per urination event (m}^2)} \times 10$$

Assuming a urine column size of 5 mm, the area per event (m^2) can be calculated based on Romera et al. (2012) as;

$$\text{Area per urination event} = \frac{\text{Average vol per event} \times \frac{1000}{10000}}{\text{Urine column} \times 0.1}$$

show that the N load per ha for cows on chicory (CHAM, CHPM) would be 185 kg UN/ha, which is 33% lower than the 285 kg UN/ha predicted for cows on RGWC. Ledgard et al. (2015) reported that a decline in the UN loading rate by 50% diminished N leaching losses from urine patches by 65%. Therefore, the decline in UN concentration from cows fed chicory is expected to diminish N leaching rates (Di and Cameron, 2007). This would reduce the environmental impacts associated with pastoral grazed dairying systems. While we acknowledge the relatively short observation period of the current study, the findings provide sufficient evidence on which to formulate further hypotheses.

Practically, the integration of chicory, or any alternative forage, should not come at the expense of milk production and quality. The results in Chapter 7 showed a significant difference in milk production between the grazing regimes studied. Chicory inclusion increased milk production and enhanced the concentration and yield of desirable polyunsaturated fatty acids in milk of cows compared with the traditional grazing regime of RGWC. Therefore, these results reflect not just the feasibility of including chicory as part of a feeding regime, but productive and potential environmental benefits.

8.5 Conclusions

The results of this study indicate a large variation in urinations and UN concentration between cows grazing RGWC only and those grazing diets containing chicory. Overall, cows on chicory-based diets increased urination frequency when compared with those on the traditional RGWC-based diets. The response in urination frequency of dairy cows grazing chicory occurred within 4-5 hours after fresh herbage allocation, regardless of timing of chicory allocation. The consistency and repeatability of the patterns of urination events observed for cows offered chicory at different times of the day provide more information about the efficiency of chicory on diurnal patterns of urinations. Urinary N concentration was substantially reduced when cows grazed chicory, regardless of time of chicory allocation. Though, there were diurnal fluctuations in UN concentrations, reflecting the different feeding regimes tested in the current study. Because of the reduction in UN concentration and change in urination behaviour, our results confirm the potential role of chicory as an alternative forage to the traditional RGWC herbage to reduce nitrogen loading and therefore promote environmentally sustainable pastoral systems.

Chapter 9

9. General discussion

9.1 Introduction

At the outset of this thesis the main aim was to investigate the effects chicory-based herbage has on milk production, milk fatty acids (FA) composition and urine N excretion of dairy cows, and to improve our understanding of the mechanisms leading to the variation in milk FA and N excretion. A secondary aim was to investigate the effect of grazing management (defoliation intensity, severity and timing) before and after vernalisation on morphology, functional traits, herbage DM production and biochemical composition of chicory. For the first time we provide the link between agronomy and dairy cow response to chicory grazing. A grazing experiment (proof of concept) was conducted to study the potential benefit of pure swards of chicory on milk FA composition and urine N excretion. The results are discussed in detail in Chapters 3 and 4. Overall, feeding chicory demonstrated potential benefits in terms of improving milk production, enhancing concentration of milk beneficial FA and reducing the concentration of N in the urine. Concurrent with animal studies, experiments on the effect of grazing management (defoliation intensity, severity and timing) of chicory on the morphology, herbage production and biochemical composition of chicory were conducted. The results are discussed in detail in Chapters 5 and 6. Based on the results obtained in Chapters 3 – 6, a second grazing experiment was conducted to test the hypotheses developed in the previous experiments. The results are discussed in detail in Chapters 7 and 8. This section will consider the overall discussion with special emphasis on the main objectives of the thesis.

9.2 Objective 1: To compare the effect of grazed chicory with traditional pasture systems on milk production and milk fatty acid composition.

9.2.1 Milk Production and composition

If chicory-based diets are to be adopted and incorporated into the grazing regime of the traditional PR diet, milk production should not be compromised. Previous studies comparing the effects of feeding chicory vs. PR on milk production of dairy cows have elicited inconsistent results. Waugh et al. (1998) and Chapman et al. (2008) noted a greater milk response from cows grazing chicory compared with those grazing PR due to increased DMI of chicory. While Muir et al. (2014, 2015) observed similar milk production responses from lactating cows offered either pure swards of chicory, chicory plus PR or pure swards of PR in spring or summer due to similarities in feeding value and DMI across treatments. To clearly elucidate the effects of feeding chicory compared with the traditional sward species, we conducted two short-term grazing experiments in summer and autumn. The question was whether feeding chicory improved milk yield?

Overall, the results from the two grazing experiments showed that feeding chicory increased milk solid production (milk fat + milk protein) when compared with the traditional RGWC. A 100% of chicory diet in late lactation and an approximately 50% chicory in mid lactation increased milk solid production by 11.2% and 11.1%, respectively, when compared with the traditional RGWC diet (Figure 9.1). The feeding value of chicory was greater than that of the traditional sward mix RGWC in both experiments, and this is probably the reason for the increase in milk production. When expressed as milk solid production per kg DM intake, treatments were similar (Chapter 3 and 4), reinforcing the advantage of feeding forages with a high nutritive value (Waghorn et al., 2007; Minneé et al., 2012). Chicory herbage has a higher ratio of non-structural to structural carbohydrates when compared with RGWC herbage, which might have improved energy supply. This premise was supported by the increased concentration of total VFA for cows on chicory diets when compared with those on RGWC-based diets (Chapter 7). Volatile FAs provide most of the energy requirement of ruminants (Bergman, 1990) and an increase in VFA is associated with increased animal performance. While chicory feeding has not consistently increased milk production in previous studies (Muir et al., 2014, 2015), no studies have reported reduced animal performances when chicory formed ≥ 20% of the diet (Minneé et al., 2017). This indicates that chicory could be incorporated into the traditional feeding regime of RGWC to maintain or enhance milk production, particularly during periods when the quality of the traditionally RGWC is low. The results in Chapter 7 further showed that offering chicory during the afternoon increased milk solid production compared with those offered chicory during the morning grazing bout. This suggest that allocating chicory herbage during the afternoon is a useful strategy that can translate to improved milk production.

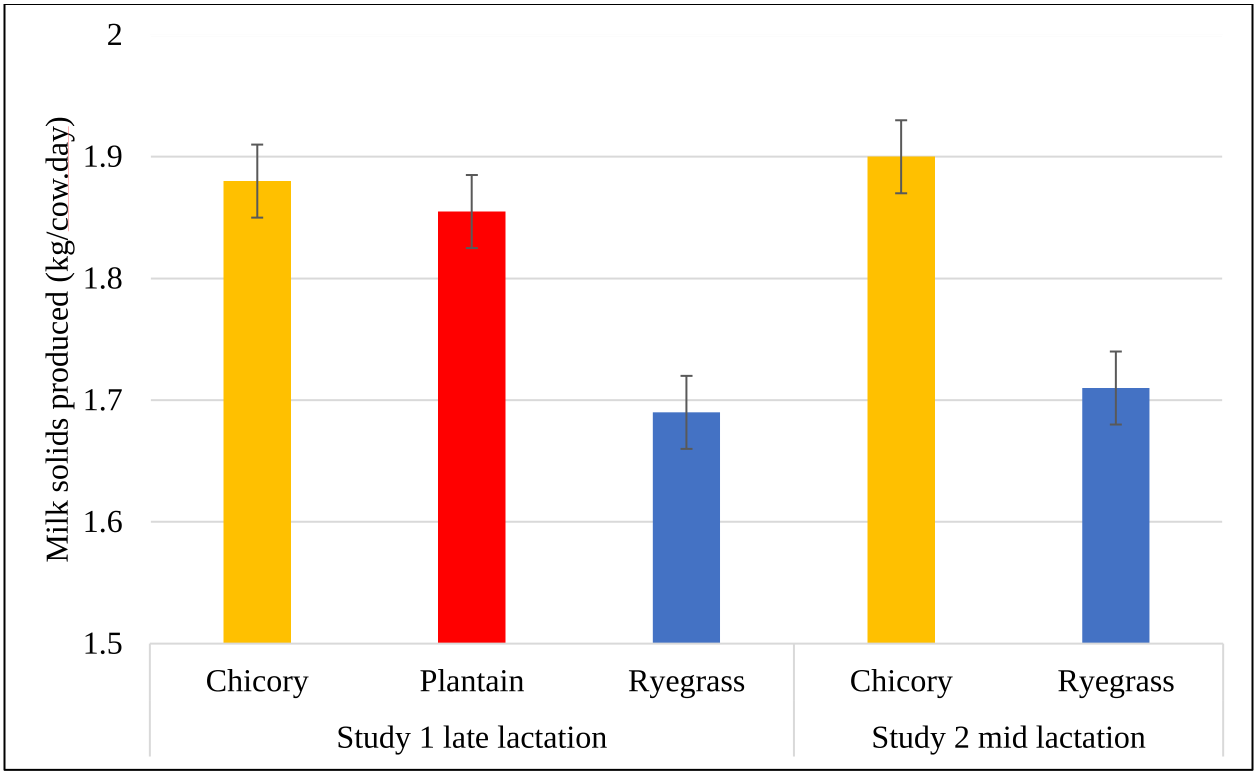

Figure 9.1. Milk solid [kg/cow.day (fat + protein)] produced from cows grazing chicory-based herbage or perennial ryegrass/white clover based-herbage during the first grazing study when chicory was vegetative and the second study when chicory was reproductive. During study 1, was fed at 100% of the diet and during study 2, chicory herbage was fed at approximately 50% of the diet. Error bars represent the standard error of the mean.

9.2.2 Milk fatty acid composition

Chicory diets significantly altered milk FA composition (Chapter 4 and 7). Chicory feeding increased the concentration of PUFA, including LA, ALA and decreased the concentration of VA and CLA. The differences in PUFA concentrations were more pronounced when chicory was vegetative (Figure 9.2). Our findings are in agreement with earlier findings which also reported enhanced concentration of PUFA from cows offered chicory-based diets compared with PR-based diets (Kälber et al., 2011; Petersen et al., 2011; Muir et al., 2014, 2015). The increase in milk LA in these studies was linked to the greater concentration of LA in the chicory herbage when compared with RGWC. However, while chicory feeding increased ALA in milk fat as compared with RGWC feeding, the corresponding swards were not generally richer in ALA (Chapter 4 and

7). This suggested that other mechanisms that improved the apparent transfer of PUFA from chicory herbage to milk are probably involved.

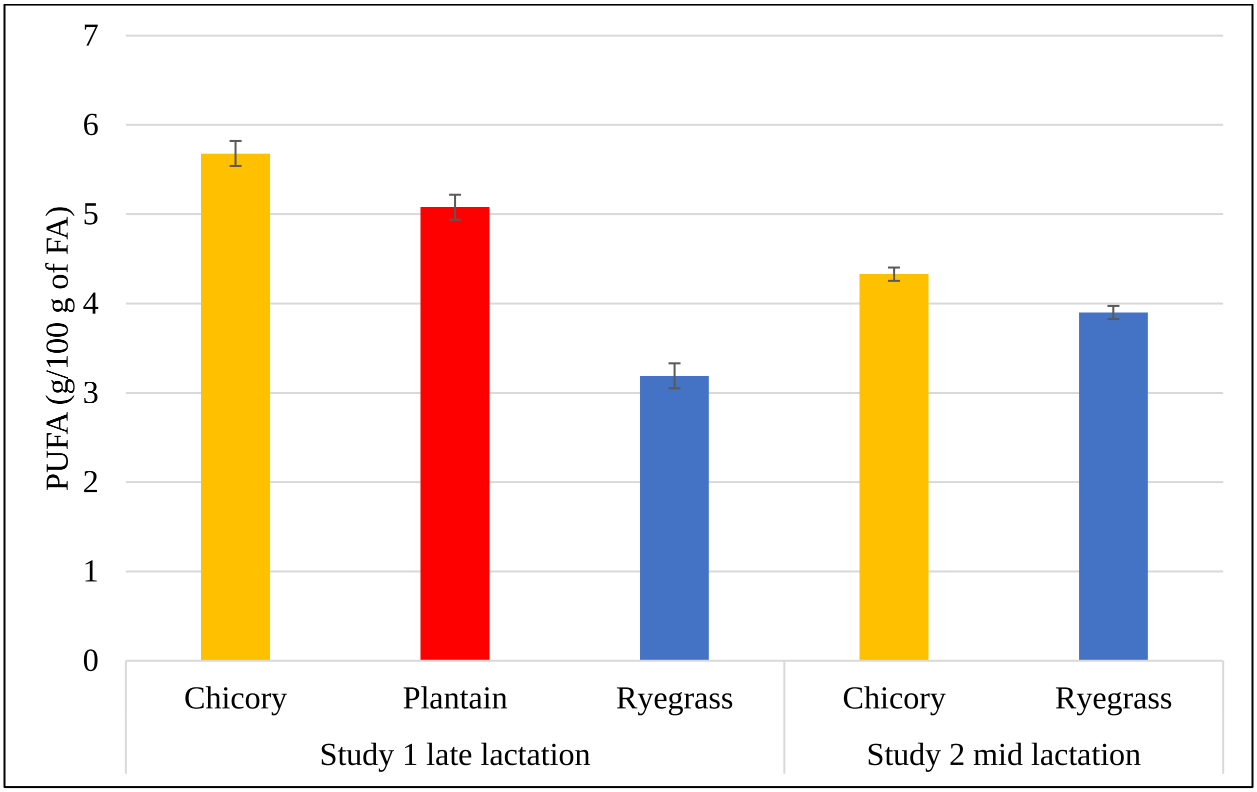

Figure 9.2. Milk PUFA concentration (g/100 g of FA) produced from cows grazing chicory-based herbage or perennial ryegrass/white clover based-herbage during the first grazing study when chicory was vegetative and the second study when chicory was reproductive. During study 1, was fed at 100% of the diet and during study 2, chicory herbage was fed at approximately 50% of the diet. Error bars represent the standard error of the mean.

To elucidate the mechanism leading to the variation in milk FA composition between cows offered chicory to those offered RGWC, we compared the effects of feeding chicory and RGWC on rumen fermentation and rumen digesta FA composition in Chapter 7. Several other studies have used differences in FA composition of rumen digesta to provide insights into rumen FA metabolism (Kim et al., 2007; Sun and Gibbs, 2012). Some evidence in Chapter 7 indicate that chicory feeding may limit lipolysis due to lower pH. Lower ruminal pH is known to inhibit the activity of lipase thus limiting lipolysis (Chilliard et al., 2007). The results in Chapter 7 showed that the mean concentrations of LA and ALA were elevated in the rumen digesta of cows grazing chicory than those on RGWC. This increase of PUFA in the rumen of cows offered the herb corresponded with reduced levels of biohydrogenation intermediate and end-product VA and stearic acid, respectively. This suggests that the level of biohydrogenation was reduced when cows grazed chicory, which increased their rumen outflow and subsequently their inter alia availability in the mammary gland, a similar premise shared by Szczechowiak et al. (2016) in milk of cows fed condensed tannins and fish-soybean oil blend mixture.

Another possible explanation for enhanced recovery of PUFA in milk of cows offered chicory could be a faster rumen passage rate as a result of reductions in microbial contact with dietary FA (Lourenço et al., 2008; Elgersma, 2015). While addressing all these questions was beyond the scope of this thesis, the decreased concentration of odd and branched-chain FA in the milk (Chapters 4 and 7) and rumen (Chapter 7) of cows grazing chicory-based diets, supports the hypothesis of reduced microbial colonization of chicory herbage than RGWC herbage, which may suggest a faster rumen passage rate of chicory herbage than RGWC. Nonetheless, the thesis confirms the potential health benefits of chicory feeing on health promoting FA, such as ALA, in ruminant products.

The increase in the concentration of ALA in milk of cows on chicory is particularly important given its human health benefits. Alpha linolenic acid exert neuroprotective, anti-inflammatory, and antidepressant properties (Blondeau et al., 2015; Nguyen et al., 2019). de Goede et al. (2011) recently reported that increased ALA intake lowered the risk of stroke. In the body, ALA is converted to eicosapentaenoic acid, a FA that is known for its cardio-protective and other human health benefits (Rajaram, 2014). In Chapters 4, and 7, feeding chicory increased the concentration of ALA in the milk by 110% and by 22%, respectively. Based on the daily milk fat yield produced by the cows on chicory and those on RGWC during the first grazing study (Chapter 4), 1.06 and 0.91 kg/cow.day, respectively, a 13.5 g difference in ALA produced per day (22.9 vs 9.4 g/cow/day) was estimated between the animals on 100% chicory and those on RGWC. While during the second grazing study, a 3.4 g difference in ALA produced per day (12.5 vs 9.1 g/cow/day) was estimated between the animals on 55% chicory and those on RGWC. If these differences are extrapolated to a herd level, assuming a commonly used stocking rate of 4 cows per hectare in the South Island of New Zealand, there would be a 54 g and 13.6 g difference on a per hectare basis per day in Chapters 4 and 7, respectively. Further, if applied to a lactation season of 305 days, there would be a 16.5 and 4.1 kg/ha per year differences in the total amount of ALA produced.

9.3 Objective 2: To compare the effect of chicory on urination behaviour and nitrogen partitioning.

9.3.1 Urination behaviour

Incorporation of forage herbs has been proposed as a means to reduce N losses from soil through altering soil N load from urination. Plantain is a high moisture, low fibre forage herb, containing plant secondary compounds and appears to alter UN excretion through changes in urea metabolism, reduced protein degradation and, perhaps most commonly, through dilution of urine (Bryant et al. 2017, Minnee et al. 2020, Nkomboni et al. 2021). From a practical perspective, additional strategies which are adoptable provide farmers with flexibility to improve their systems by reducing nutrient losses. Greater flexibility and adoption of mitigations might be achieved by identifying several plants species which have the beneficial features and their mode of action for reduced urinary N load. However, the current research has focused on the role of plantain with little emphasis on the alternative herb chicory. Chicory is known to have high palatability and healthy fatty acid (**FA**) profile, but little is known about its effect of water metabolism and urine excretion patterns. Chicory has the attributes required to reduce the concentration of UN. For example, chicory has a higher moisture content than RGWC; a trait that has been identified as a means to reduce UN load and subsequent nitrate leaching from dairy cattle grazing in pastoral systems (Bryant et al., 2019).

This is the first thesis to investigate the urination patterns of dairy cows grazing chicory and comparing them with plantain and RGWC. Chapters 3 and 8 demonstrated that chicory feeding increased the urination frequency and daily urine volumes when compared with RGWC. A summary of the results is shown in Figure 9.3. Pearson's correlation analysis in Chapter 3 demonstrated that urine volume was strongly correlated with feed water ($R = 0.81$; $P < 0.05$), sodium ($R = 0.65$; $P < 0.01$) and potassium ($R = 0.70$; $P < 0.01$). Chicory has high moisture and mineral contents, factors that have been shown to increase urine volume in ruminants (Dijkstra et al., 2013; Bryant et al., 2019).

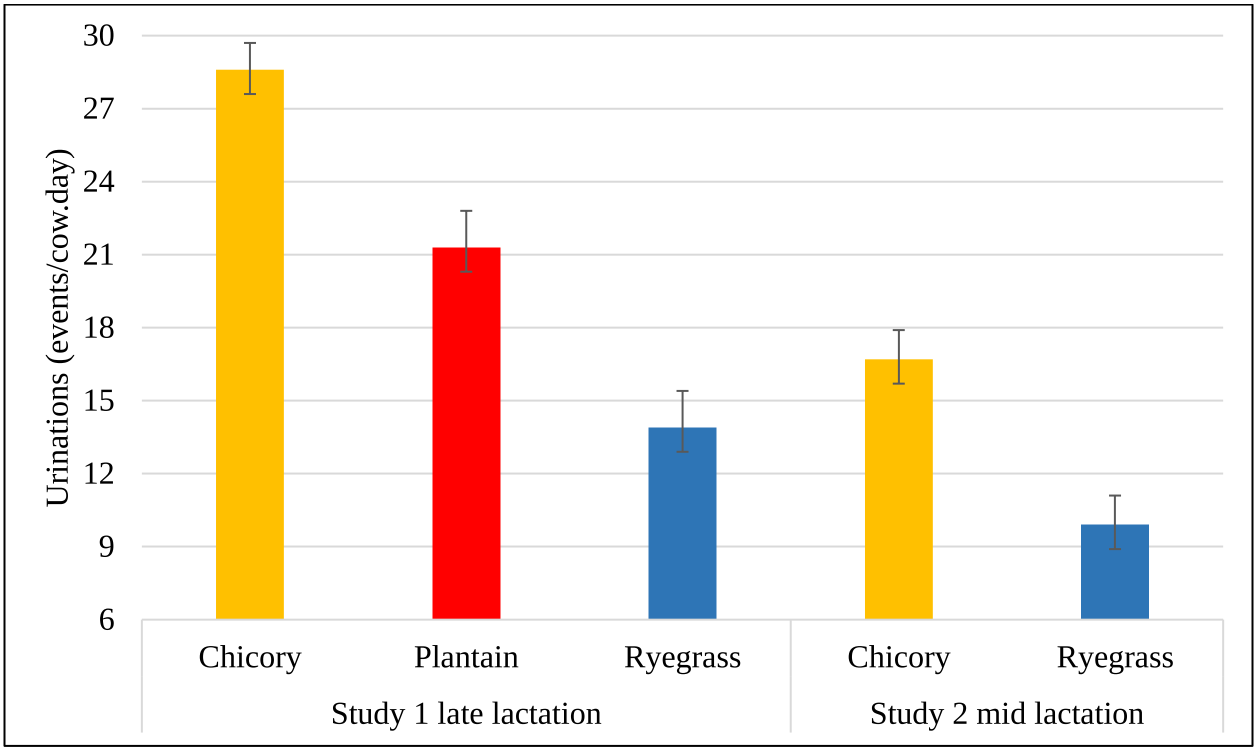

Figure 9.3. Urination patterns (events/cow.day) of cows grazing chicory-based herbage or perennial ryegrass/white clover based-herbage during the first grazing study when chicory was vegetative and the second study when chicory was reproductive. Error bars represent the standard error of the mean.

9.1.1 Nitrogen partitioning

Urinary N concentration declined by 74% and 33% when 100% or approximately 50% of chicory was included into the RGWC diet of lactating dairy cows in autumn and summer, respectively (Chapters 3 and 8). A decline in UN concentration in cows offered chicory diets has previously been reported in New Zealand (Totty et al., 2013; Bryant et al., 2017; Minneé et al., 2017). This was regardless of the similar N intakes between RGWC or chicory-based diets. There are two plausible explanation for the differences in urinary N excretion between cows offered chicory and those offered RGWC, with the first one being increased urine volume from cows fed high moisture diets which include herbs such as chicory. Increases in urine volume dilute the concentration of N in the urine. This explanation is in line with previous studies which also observed reductions in UN concentrations from cows fed diuretics such as salt (Spek et al., 2012; Ledgard et al., 2015) or high moisture forage plantain (Box et al., 2017a). The second explanation could be the differences in NUE, which was greater for cows on chicory diets (Chapters 3 and 8). The improvements in NUE of cows fed chicory is further confirmed by the lower concentration of MUN, a biomarker of the efficiency for milk production (milk N/N intake) in lactating cows (Huhtanen et al., 2015). At similar N intake, improvements in NUE and reductions in UN concentration suggest that N partitioning was altered in cows offered chicory, a similar premise shared by Totty et al. (2013) for cows offered diverse diets containing chicory. Chicory forage has a higher ratio of non-structural carbohydrates to crude protein than RGWC (Chapters 3, 4, 6, 7 and 8) which might have improved energy and protein supply to meet microbial requirements and improved milk protein daily yields and NUE (Edwards et al., 2007).

The concentration of UN has been found to have major impacts on the environment. The risks of nitrate leaching at urine patches depend on N load, which is a function of urine volume and concentration of UN (Li et al., 2012). While this thesis did not investigate N leaching rates, extrapolations based on the equations developed by Selbie et al. (2015) to estimate the average UN loading rate;

$$\text{UN rate (kg UN ha}^{-1}) = \text{Conc (g UN L}^{-1})\,\frac{\text{Vol (L)}}{\text{Area per urination event (m}^{2})} \times 10$$

Assuming a urine column size of 5 mm, the area per event (m^2) can be calculated based on Romera et al. (2012) as;

$$\text{Area per urination event } = \frac{\text{Average vol per event} \times \frac{1000}{10000}}{\text{Urine column} \times 0.1}$$

show that the N load per ha for cows on 100% chicory would be 64 kg UN/ha, which is 73% lower than the 242 kg UN/ha for cows on RGWC during the first grazing study. While during the second grazing study where chicory cows consumed ~50% chicory in their daily diet, the N load per ha for cows on ~50% chicory would be 185 kg UN/ha, which is 33% lower than the 285 kg UN/ha for cows on RGWC. Ledgard et al. (2015) reported that a decline in the UN loading rate by 50% diminished N leaching losses from urine patches by 65%. Therefore, the decline in UN concentration from cows fed chicory is expected to diminish N leaching rates (Di and Cameron, 2007). This would reduce the environmental impacts associated with pastoral grazed dairying systems. While we acknowledge the relatively short observation period of the studies reported in this thesis, the findings provide sufficient evidence on which to formulate further hypotheses.

9.4 Objective 3: To investigate the effect of defoliation interval, severity and time of day on agronomic and chemical response of chicory.

As discussed, it is apparent that chicory presents a number of animal, product and environmental benefits, yet concerns over second season reproductive stem and its limited persistence have slowed the adoption of the herb in pastoral systems (Li and Kemp, 2005; Lee et al., 2015a). In order to generate efficient and specific defoliation strategies of chicory pastures that could be used to control production and feeding value of chicory on farm, Chapters 5 and 6 were designed to investigate the effect of defoliation interval, severity and time of day on the morphology, functional traits, herbage production, nutrient composition and FA composition of chicory herbage before and after vernalisation.

9.4.1 Morphology and functional traits

9.4.1.1 Below ground

Overall, the findings of this research showed considerable defoliation interval effect on the morphology of chicory plants before and after vernalisation (Chapter 5). As expected, the size of the taproot and its concentration of WSC was consistently greater for plants managed under longer defoliation intervals than those managed under shorter defoliation intervals across the two phenological stages. Results on root development dynamics across seasons and in different growth stages in chicory or other herbs are scarce, but the patterns reported in the current study are in agreements with the patterns reported in other herbaceous plants such as perennial and annual ryegrass plants (Donaghy and Fulkerson, 1998; Solomon et al., 2017). After a defoliation event, the growth rate of the plant increases, utilising the stored reserves in the roots (Briske and Richards, 1995; Donaghy and Fulkerson, 1997). Lee et al. (2015b) showed that pre-defoliation levels of carbon reserves in the taproots of chicory were attained after 310 GDD of regrowth. By delaying defoliating chicory to 600 GDD in the present study, larger amounts of reserves were stored in the roots, increasing the root size and their concentration of WSC. The resulting decline in root WSC reserves with frequent defoliation (at 300 GDD) might compromise the longevity of the plants.

While the greater concentration of WSC in roots of 600 GDD plants may explain the increased herbage mass compared with 300 GDD plants as reported in Chapter 5. Stored carbohydrates play a vital role in the growth and development of forages in temperate regions and their increased concentration is associated with greater forage yield (Solomon et al., 2017).

9.4.1.2 Above ground biomass

In general, the number of vegetative shoots in herbaceous plants is reported to be greater in plants managed under shorter defoliation intervals (Briske and Richards, 1995). Except for the time before vernalisation, this was found to be true in the current study, as chicory plants managed under the shorter regrowth interval had more vegetative shoots than those managed under the longer regrowth interval after vernalisation. The lack of variation in the number of shoots and leaves per plant before the plants were vernalised, whatever the management practices were applied (Chapter 5) suggest that the regrowth intervals used in the current study did not significantly affect the above ground, an observation that corroborates earlier findings (Li et al., 1997c; Clapham et al., 2001). While the increase in vegetative shoots in frequently defoliated plants (3 – 4 weeks interval in spring and summer) is in agreement with Li et al. (1994) who also reported a flush of vegetative shoots during the growing season in chicory plants defoliated every 3–5 weeks. While an increase in reproductive stems of perennial herbaceous plants may increase the accumulated herbage mass, as shown in Chapter 5, mature reproductive stems decrease forage quality due to a lowered leaf-to-stem ratio within individual plants and the more structural carbohydrates in mature stems (discussed in detail in Chapter 6). Therefore, specific grazing management regimes should be designed to control the growth and development of reproductive stems in winter vernalised chicory.

A subsequent study investigated the effect of phenological growth stage on leaf and stem development dynamics of vernalised chicory plants in relation to thermal time. The thermal time to initiate stem elongation in plants managed under longer intervals was ~274 growing degree-days. This result should be considered when designing management practices of chicory. While previous research has used fixed number of calendar days to control the growth of reproductive stems in chicory, as reviewed by Li and Kemp (2005), the findings from this thesis provide a management directive for timing of defoliation of chicory in order to maintain feed quality for grazing livestock. Further research is needed to explore whether alternating frequent and infrequent defoliation regimes during different seasons or phenological stages may optimise vegetative growth, root reserves, and pasture persistence.

9.4.2 Herbage production

Herbage production of herbaceous plants is highly dependent on plant population (Li and Kemp, 2005), plant size (Li et al., 1997a) and root WSC concentration (Lee et al., 2015b; Solomon et al., 2017), and as the findings in Chapter 5 show similar plant populations across treatments during the first 16 months, the variation in herbage production could be explained by differences in plant size and/or root WSC concentrations. Contrastingly, defoliation height did not have much of an effect on herbage DM production. Several other studies have reported minor effects of defoliation height on herbage DM production of chicory (Labreveux et al., 2004; Lee et al., 2015a). Combined, these findings confirm that chicory responds more to defoliation frequency than it does to defoliation height by altering root WCS concentration and individual plant biomass.

A plethora of defoliation strategies based on fixed number of calendar days or plant height as a criterion for when to defoliate chicory and their effects on cumulative herbage mass have been evaluated (Clark et al., 1990a; Jung et al., 1996; Li et al., 1997a; Labreveux et al., 2004; Lee et al., 2015a). Regardless of which defoliation criterion was used in these studies, chicory plants defoliated after longer intervals accumulated greater herbage mass than those defoliated after shorter intervals. The studies in this thesis used cumulative GDD as a criterion for when to defoliate chicory, and the effects on herbage production were recorded. In agreement with previous experiments, the herbage produced was found to be greater in plants exposed to longer defoliation intervals after vernalisation than those exposed to shorter regrowth intervals (8.7 vs. 7.7 ± 0.49 kg DM/ha per GDD).

In high producing intensive pastoral dairying systems, grazing management practices should consider herbage DM production, swards species persistence and feeding value of the herbage. The total herbage yield of chicory (at 110 kg N/ha/year fertiliser rates) in its first year following establishment (February 2018 – February 2019) ranged between 14.5 – 16.1 t DM/ha in the present study. These are a little lower than those of the conventional RGWC pasture yields from the same site of 16.5 (at 150 kg N/ha/year fertiliser rates) and 18.0 t DM/ha (at 400 kg N/ha/year fertiliser rates) (Chapman et al. 2020), but comparable with the yield range of 14.3 t DM/ha – 15.3 t DM/ha reported for first year chicory defoliated at 3–5 weeks between March 2011 and May 2012 in Hamilton, New Zealand (Lee et al., 2015a).

9.4.3 Chemical composition

Balancing herbage production and nutritional value of forages in grazed dairying systems does not always align. An increase in herbage production is usually accompanied by a decline in feeding value of the herbage (Lee et al., 2015a; Chen et al., 2019; Ta et al., 2020). This was found to be true in the current study, as plants exposed to longer defoliation intervals increased aerial biomass but reduced the nutritional value of the herbage after vernalisation. The nutritional composition of chicory is highly dependent on stage of maturity, N fertiliser rates and defoliation interval, factors that influence the proportion of leaf material in the herbage. Li and Kemp (2005) recommended that grazing management strategies should be designed to maintain a desirable 30:70 stem to leaf ratio. As the leaf to stem ratio was greater in plants exposed to shorter defoliation intervals (55 – 62% vs 31 – 35%), it is not surprising that the nutritional value of plants under the shorter regrowth interval was greater than those exposed to longer regrowth intervals. The results confirm the important role of grazing management to control the growth and development of stems, and therefore, the feeding value of chicory herbage.

The dietary CP requirement of dairy cows ranges between 140 to 180 g/kg of DM which is strongly linked with stage of lactation (NRC, 2001). The low values for CP reported in Chapter 6 (≤ 132 g/kg CP of DM) after vernalisation are likely to limit milk production in early and mid-lactation. This is especially true for chicory herbage under a longer defoliation interval which showed a CP concentration ranging between 75 – 910 g/kg CP of DM. However, in the grazing experiment reported in Chapter 7, in which the CP concentration of the herbage ranged between 127 – 157 g/kg of DM, the low CP did not appear to limit milk production because the cows selected the leaf material over the stem material. Allocation of chicory herbage high in stem content should consider likelihood of stock refusal of stem and attempt to avoid underfeeding to achieve acceptable animal performance levels.

Diurnal changes in nutritive composition revealed, irrespective of vernalisation status, greater WSC and NFC concentrations in herbage harvested in the afternoon, but lower CP and fibre concentrations (Chapter 6). Such increases in the ratio of readily fermentable carbohydrates to CP have previously been used in other forages to improve energy and protein supply to meet microbial requirements and improve NUE (Kebreab et al., 2001). This could also be beneficial in pastoral systems as improvements in NUE could reduce N loss via urine thus diminishing the environmental impacts associated with the traditional feeding systems (Edwards et al., 2007). In a subsequent grazing study (Chapter 7), we compared the effects of morning or afternoon allocation of chicory on milk production. This allowed us to evaluate the potential benefits of the diurnal changes of herbage chemical composition on milk production. The results are discussed in section 9.2.

9.4.4 Fatty acid composition

Another aim of this thesis was to examine the FA concentration of chicory herbage under different management regimes (defoliation interval, severity and timing) before and after vernalisation. Fatty acids, which are the main form of lipids in the herbage, are the main component of biological membranes (Kim, 2020). They are used a reserves of carbon and energy in triacylglycerol and regulators of stress signalling (He and Ding, 2020). Several studies have shown that greater concentrations of unsaturated FA are found in leaf material than stem material. The findings from the current study demonstrated considerable phenological and defoliation interval effects on FA concentration of chicory herbage. As with the nutritional composition, the concentration of FA in the herbage declined with advancing phenological. Results on FA content of chicory at different growth stages are scarce, but the pattern of change observed in this thesis in in agreement with observations from other herbaceous plants (Boufaïed et al., 2003). Extending defoliation interval after vernalisation reduced the herbage concentration of LA, ALA and total FA by 28%, 40% and 33%, respectively. The common basis for losses of FA in herbaceous plants with longer regrowth intervals in the previous studies was the increased proportion of stem material in the herbage. The FA concentrations in stem material of herbaceous plants is 50% to 66% less than the concentrations found in leaves (Boufaïed et al., 2003). This may partially explain the decline in FA concentrations of herbage managed under longer regrowth intervals. Together with the results in the literature, our findings suggest that the leaf to stem ratio is vital in determining the concentration of herbage

FA and highlights the importance of agronomic practices on FA composition of chicory herbage after vernalisation.

While LA and ALA are extensively biohydrogenated in the rumen, it has been often found that their concentration in milk of ruminants is influenced by their concentration in the herbage (Jenkins et al., 2008; Elgersma, 2015; Toral et al., 2018). As a result, it is strongly desirable to design management practices that would enhance their concentration in the herbage in order to positively influence the PUFA content of milk. Our data suggest that shorter defoliation intervals will probably increase the levels of Omega-3 FA and CLA in milk of ruminants as compared with longer defoliation interval due the increased concentration of their precursors in the herbage. However, this requires verification in future studies as other factors such as increases in secondary compounds during maturing of chicory plants could play a role lipids transfer efficiency from herbage to milk (Kälber et al., 2014).

There was some evidence for diurnal fluctuations on some individual FA as increases in the concentrations of oleic acid and LA increased from morning to afternoon herbage by an average of 24.2% and 22.3%, respectively, after vernalisation were observed (Chapter 6). The greater concentration of LA in afternoon herbage is expected to positively influence the CLA concentration in the milk. In the subsequent grazing study (Chapter 7), we compared the effects of morning or afternoon allocation of chicory on milk FA composition. This allowed us to evaluate the potential benefits of the diurnal changes of herbage FA composition on milk FA composition. The results are discussed in section 9.4.

9.5 Overall conclusions

a. With the increase in consumers interest in quality of their food, the concentration of specific milk FA is becoming increasingly important. Including chicory into the traditional feeding regime of RGWC could enhance the concentration of functional FA in ruminant-source foods FA such ALA by improving transfer efficiency of herbage FA by reducing the extent of biohydrogenation in the rumen.

b. This thesis explored the novel concept of management in grazed dairying systems which offers a foundation for other research exploring possibilities to reduce N loss in pastoral systems by including high moisture chicory into the traditional feeding regime of RGWC. Losses of N to the environment is a major issue in pasture-based livestock production systems in temperate regions. The incorporation of high moisture chicory could mitigate the loss of N by dilution of urinary N through increased urination frequency and by improving the synchrony of energy with N, ultimately improving NUE and reducing total N excreted in the urine.

c. This thesis confirmed the challenges of balancing between herbage production and feeding value of forages under managed grazing systems as chicory plants exposed to longer regrowth intervals accumulated larger amounts of aerial mass and reduced the highly nutritious leaf proportion of the herbage. Alternating frequent and infrequent defoliation regimes might be used to optimise vegetative growth, root reserves, and pasture persistence.

d. A key finding from the thesis quantified the growing degree-days to initiate stem elongation post vernalisation, which provides management directive for timing of defoliation of chicory in order to maintain feed quality for grazing livestock.

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